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Aminomethyltetrazoles as potential inhibitors of the γ-aminobutyric acid transporters mGAT1–mGAT4: Synthesis and biological evaluation

Eva S. Schaffert, Georg Höfner, Klaus T. Wanner *

Department of Pharmacy, Center for Drug Research, Ludwig-Maximilians-University Munich, Butenandtstr. 5-13, D-81377 Munich, Germany

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ABSTRACT

1,5-Disubstituted and 5-monosubstituted aminomethyltetrazole derivatives derived from glycine were synthesized employing a $TMSN_3$-modified variant of the Ugi reaction as a key step. All compounds were evaluated regarding their inhibitory potency and subtype selectivity at the four murine GABA transporter subtypes mGAT1–mGAT4. Though none of the 5-monosubstituted tetrazoles turned out to inhibit [^{3}H]GABA uptake to a significant extent, the 1,5-disubstituted tetrazole derivatives displayed a distinct activity, especially at the GABA transport proteins mGAT2–mGAT4. Thus, a reasonable potent and selective inhibitor of mGAT3 was found. Additionally, two more compounds were identified as potent inhibitors of mGAT2. This is especially relevant, as up to date only few potent inhibitors of mGAT2 that do not affect mGAT1 are known.

1. Introduction

Brain function is based on a fine-tuned balance of excitatory and inhibitory signals that is maintained by numerous feedback-mechanisms.[1] An imbalance of excitation and inhibition may underlie numerous neuropathological and psychiatric diseases of the central nervous system (CNS). With approximately 30–40% of all synapses being GABAergic, γ-aminobutyric acid (GABA, **1**, Fig. 1) is the major inhibitory neurotransmitter in the mammalian brain.[2,3] Thus, perturbations in GABA neurotransmission play a key role in the pathophysiology of neurological disorders. For example, a deficiency of GABA is related to epilepsy,[4] Morbus Parkinson,[5] Morbus Alzheimer,[6] Huntington's chorea,[7] neuropathic pain,[8] schizophrenia,[9] and depression[10]—to name only a few.

Consequently, virtually all receptors, metabolic enzymes and transporters involved in GABAergic neurotransmission can be considered as valid targets when designing new CNS-active drugs. For example, enhancement of the GABAergic signal transduction can be effected either by direct receptor agonism or allosteric modulation of the GABA receptors, as it is accomplished by benzodiazepines or barbiturates.[11] On the other hand, the GABAergic neurotransmission can be enhanced by increasing the concentration of GABA in the synaptic cleft. This may be achieved by inhibition of enzymatic GABA degradation or by blocking specific high affinity GABA transport proteins (GATs) responsible for the removal of synaptic GABA.[11] These GABA transporters are located in the cell membranes of the presynaptic nerve terminals and also in those of glial cells. The genes encoding the GABA-uptake proteins have been identified as members of the SLC6 (solute carrier 6) family. Genes coding for other Na^+/Cl^- dependent transporters, such as the transport proteins for serotonin, dopamine, norepinephrine or glycine, have also been assigned to this group.[12,13]

To date, four different subtypes of membrane bound GABA transport proteins are known.[14] When cloned from murine brain cells these GABA transporters are termed mGAT1, mGAT2, mGAT3, and mGAT4. The nomenclature suggested by the Human Genome Organization (HUGO) as well as the nomenclature for GATs originating from other species including humans and rats, however, differs from the mouse nomenclature. Here, the corresponding GABA transport proteins are denoted as GAT-1, BGT-1, GAT-2, and GAT-3, respectively.[†,14]

The four subtypes of GABA transporters differ in their expression pattern and their pharmacological role. Whereas mGAT2 and mGAT3 are also found in peripheral tissue, the most prevalent GABA transporter subtypes, mGAT1 and mGAT4, are almost exclusively located in the CNS.[15,16] mGAT1 is widely distributed throughout the brain with high densities found for example in the neocortex, hippocampus, cerebellum, and the basal ganglia.[13,16] The distribution of mGAT4, however, is more restricted.[14] It has a strong expression in the spinal cord, the brain stem, the thalamus, and hypothalamus.[16] mGAT3 has been found to be primarily located on the leptomeninges and in ependymal tissue.[17] The mRNA encoding mGAT2, was found mainly in the hippocampus, hypothalamus, thalamus, substantia nigra, and the amygdala.[15]

* Corresponding author. Tel.: +49 89 2180 77249; fax: +49 89 2180 77247.
E-mail address: klaus.wanner@cup.uni-muenchen.de (K.T. Wanner).

† In the present paper mouse nomenclature will be used, as the biological characterization of the test compounds was performed using murine GABA transporters.

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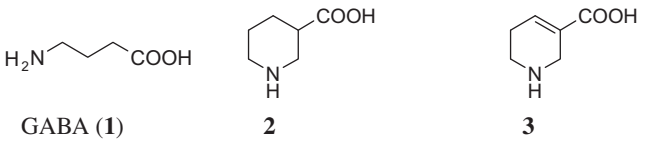
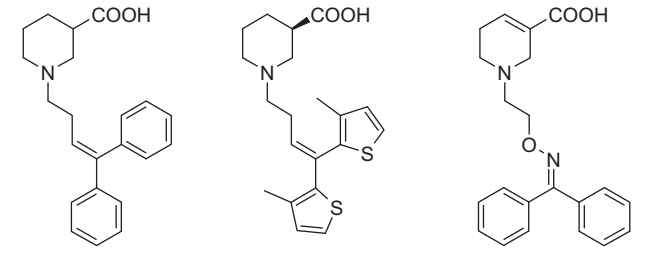


GABA (**1**) **2** **3**

Figure 1. GABA (**1**) and conformationally restricted GAT inhibitors.

While mGAT2 and mGAT3 are predominately located at extrasynaptic sites,[15,16] mGAT1 and mGAT4 have been found in close proximity to the synapse or in the synapse itself. Thereby, mGAT1 is mainly assigned to neuronal transport, while it is assumed that mGAT4 accounts predominantly for glial transport of GABA.[16,18] Except for mGAT2, the GABA transport proteins show high affinity to GABA. mGAT2 possesses only low affinity, but is also able to carry betaine.[16,19]

Since the identification of the GABA transport system as pharmacological target[20,21], a number of acyclic and cyclic structures have been tested as potential GAT inhibitors. Inhibitors of the first generation, mainly analogs of GABA (**1**) itself, often showed low selectivity for the GABA transporters.[22] The conformationally restricted amino acids nipecotic acid (**2**) and guvacine (**3**, Fig. 1) were eventually found to be potent inhibitors of [^{3}H] GABA uptake demonstrating high in vitro GABA uptake inhibition without intrinsic activity at $GABA_A$-receptors.[23] Being relatively small, at physiological pH zwitterionic molecules, these first GABA uptake inhibitors, however, proved to be too polar to cross the blood–brain barrier in sufficient quantities. For this reason, some 30 years ago the first lipophilic derivatives of nipecotic acid and guvacine have been developed.[24] Addition of characteristic lipophilic side chains to the parent compounds resulted in potent GABA uptake inhibitors such as SK&F-89976-A (**4**),[24] Tiagabine (**5**),[25,26] or NO-711 (**6**)[27] (Fig. 2). These compounds were not only found to be systemically active and considerably more potent than the parent compounds, but also selective for the GABA transporter subtype mGAT1.

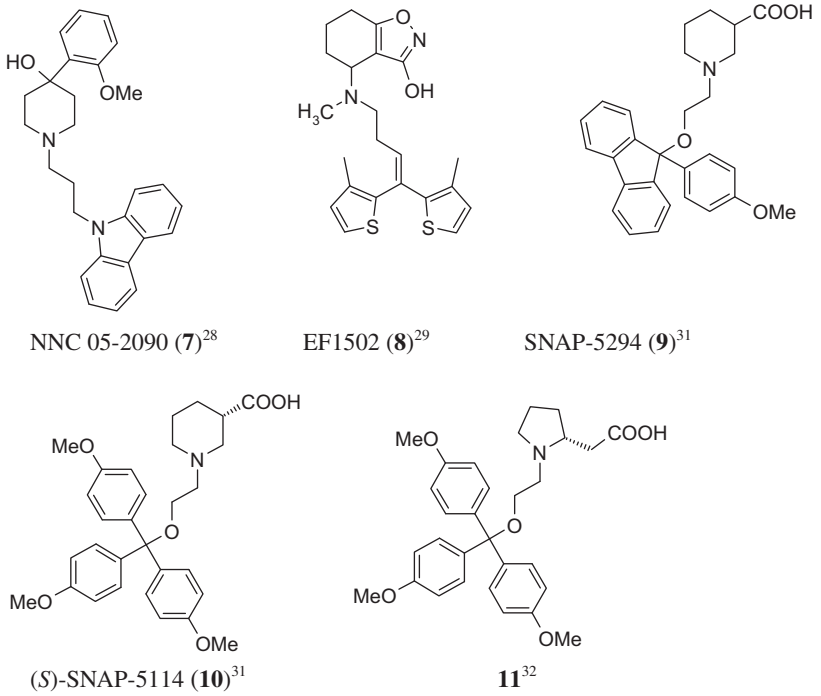


SK&F-89976-A (**4**)[24] Tiagabine (**5**)[25,26] NO-711 (**6**)[27]

Figure 2. mGAT1 selective GABA uptake inhibitors.

Up to now, the majority of published GAT inhibitors target the transporter subtype mGAT1. The number of effective inhibitors of the other subtypes, however, is still small. A closer look at two of the most potent mGAT2 inhibitors known to date, NNC 05-2090 (**7**)[28] and the mGAT1/mGAT2 selective EF1502 (**8**),[29] reveals that neither is derived from a classical heterocyclic amino acid parent structure (Fig. 3). Detailed pharmacophore models for mGAT2 inhibitors, however, are still lacking. For mGAT3 and mGAT4, too, only few selective inhibitors have been published. Small amino acids like β-alanine or (*RS*)-2,3-diaminopropionic acid are reported to exhibit moderate affinity to GABA uptake transporters with selectivity for mGAT3 and mGAT4.[30] As for the lipophilic inhibitors of mGAT3 and mGAT4, for example SNAP-5294 (**9**),[31] or the mGAT4 selective inhibitors (*S*)-SNAP-5114 (**10**)[31] and compound **11**,[32] which is derived from the only moderately potent (*S*)-pyrrolidine-2-yl-acetic acid, subtype selectivity seems to originate mainly from the structure of the lipophilic side chain attached to the nitrogen of the parent structure (Fig. 3).

With Tiagabine (**5**) in clinical use for the therapy of partial seizures, mGAT1 represents an approved drug target.[33] Yet, little is known about the possible therapeutic relevance of mGAT2–mGAT4. Potent inhibitors of these GABA transporter subtypes are of fundamental importance for the elucidation of the physiological function of these targets and, possibly, for the development of a novel class of CNS-active drugs.[19]



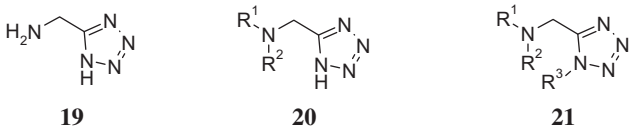
NNC 05-2090 (**7**)[28] EF1502 (**8**)[29] SNAP-5294 (**9**)[31]

(*S*)-SNAP-5114 (**10**)[31] **11**[32]

Figure 3. Selected inhibitors of mGAT2–mGAT4.

As part of a research project aimed at the development of tetrazole analogs of amino acids as new inhibitors of the GABA transport proteins mGAT1–mGAT4, a series of glycine-derived 5-aminomethyltetrazoles was synthesized. Bioisosteric replacement of functional groups is a widely used technique in medicinal chemistry for the generation of more potent, more selective or better tolerated analogs of lead structures.[34] Thereby, the 1*H*-tetrazole moiety represents a popular substitute for carboxylic acids. Yet, little is known about the effect of tetrazole analogous amino acids on GABA uptake. In a report published as early as 1984, five tetrazole amino acids (**13**–**17**), including the analogs of nipecotic acid (**2**), GABA (**1**) and β-alanine (**12**), the compounds **13**, **17** and **15** have been tested regarding their impact on the GABAergic system (Fig. 4). Their potency to inhibit the [^{3}H]GABA uptake into synaptosomes from rat brain, however, was low (IC_{50} <100 μM).[35]

To the best of our knowledge, the influence of other tetrazole amino acids on GABA uptake, as well as the effect of their lipophilic derivatives is still unknown. Thus, it seemed worthwhile to further investigate tetrazole analogs of various amino acids with regard to their impact on the GABA transport system. As starting point for the present study, glycine (**18**) was chosen. Glycine (**18**) is an inhibitor of the non-GAT1 transporters with only low activity (pIC_{50} mGAT2 2.65 ± 0.10, mGAT3 2.88 ± 0.02, mGAT4 2.51 ± 0.12) and modest selectivity as compared to mGAT1 at which it is devoid of any significant potency (59.8%, 10 mM, see Table 1). Aim of this study was to investigate the influence of bioisosteric replacement of the carboxylic acid by the larger and more lipophilic 1*H*-tetrazole on the activity of glycine (**18**) and its derivatives at the GABA transport proteins. Here, we present the synthesis of the glycine analog 1*H*-tetrazol-5-ylmethylamine (**19**) and of several lipophilic derivatives (general structure **20**), as well as their biological evaluation regarding their inhibitory potency and subtype selectivity at the four murine GABA transport proteins mGAT1–mGAT4. Additionally, the 1,5-disubstituted tetrazole derivatives of the general structure **21** were also included in the present study (Fig. 5).



19 **20** **21**

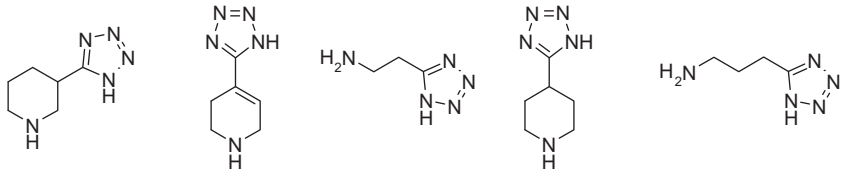
Figure 5.

2. Results and discussion

2.1. Chemistry

For the assembly of the aminomethyltetrazole-structure, a $TMSN_3$-modified variant of the Ugi reaction was chosen. The term Ugi reaction actually comprises a group of multi-component reactions generally involving a carbonyl compound, an amine, an isonitrile and an acid component. Thereby, the nature of the components may vary widely.[36] When employing an azide salt, or, more conveniently, trimethylsilyl azide as azide source, 1,5-disubstituted α-aminotetrazole derivatives are obtained.[37–41]

Thus, starting from paraformaldehyde (**36**) as carbonyl component, this variant of the Ugi reaction offers direct access to the 1,5-disubstituted α-aminomethyltetrazoles of the general structure **21** (Scheme 1). By employing appropriate substituted amines and isonitriles, assembly of the aminomethyltetrazole and linkage with lipophilic groups is possible in only one step. Cleavage of a suitable protecting group in 1-position of the tetrazole ring eventually leads to the amino acid analogs of the general structure **20**. For this purpose, we chose the 1,1,3,3-tetramethylbutyl (tmb)-group, which has been described as a convenient protective group for the tetrazole moiety.[42]



13 **14** **15** **16** **17**

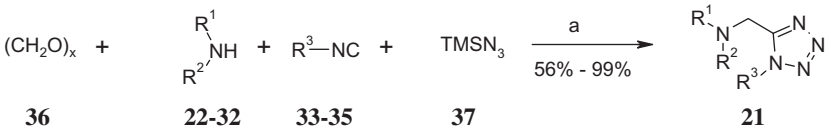
Figure 4.

Table 1
Test results of selected reference compounds

Entry	Compound	mGAT1	mGAT2	mGAT3	mGAT4
1	H_2N COOH	59.8%[a] (10 mM)	2.654 ± 0.097[a]	2.884 ± 0.023[a]	2.506 ± 0.118[a]
	18	83.1%[b] (1 mM)	60.7%[b] (1 mM)	49.3%[b] (1 mM)	69.7%[b] (1 mM)
2	H_2N COOH	2.59 ± 0.03[a]	3.48 ± 0.11[a]	4.66 ± 0.06[a]	4.46 ± 0.13[a]
	12				
3	NNC 05-2090	4.99 ± 0.06	5.09 ± 0.06	4.93 ± 0.03	4.78 ± 0.10
	7	(4.72)[c]	(5.85)[c]	(4.39)[c]	(4.82)[c]
4	(*S*)-SNAP-5114	4.07 ± 0.09[d]	56% (100 μM)[d]	5.29 ± 0.04[d]	5.81 ± 0.10[d]
	10	(3.41, hGAT-1)[e]	(3.85, hBGT-1)[e]	(4.68, rGAT-2)[e]	(5.30, hGAT-3)[e]

If not stated otherwise, the results are given as pIC_{50} ± SEM of three independent experiments, each performed in triplicate. Percentages represent specific binding remaining in presence of the inhibitor concentration given in parentheses. To allow a better comparison, all pIC_{50} values given have been determined employing our test system (see Ref. 52).

[a] From Ref. 53; data determined employing our test system.
[b] These data are given for a more convenient comparison with the test results presented in Table 2.
[c] Data reported by Thomsen et al., using a protocol differing from our test system (see Ref. 28).
[d] From Ref. 52; data determined employing our test system.
[e] Data reported by Dhar et al., using a protocol differing from our test system (see Ref. 31).



36 22-32 33-35 37 21

Scheme 1. Reagents and conditions: (a) MeOH, 80 °C (MW), 1.5 h.

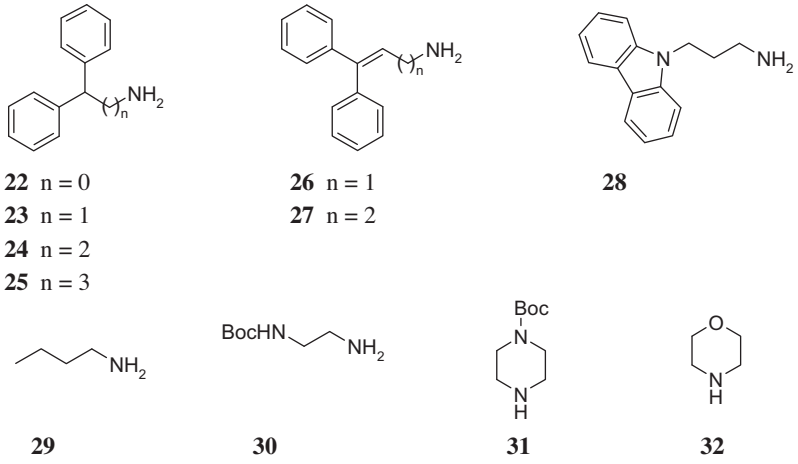


Figure 6. Amine components employed in the $TMSN_3$-modified Ugi reaction.

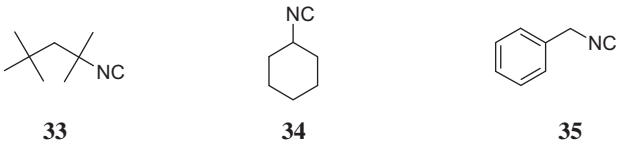


Figure 7. Isonitrile components employed in the $TMSN_3$-modified Ugi reaction.

The focus was on amino acid analogs that are substituted with lipophilic groups which have been identified to be beneficiary for the pharmacokinetic profile as well as for the binding to GAT-proteins. Hence, primarily the amines **22**–**28** bearing bis-aromatic structures were chosen. By variation of the spacer length, the optimum distance between the amino function and the aromatic moiety should be determined.

Additionally, four more primary and secondary amines **29**–**32** were employed in the Ugi reaction, to investigate the influence of a pure alkyl chain, additional rings and further functional groups on GAT inhibition. In case of the diamines **30** and **31**, the mono-Boc-protected form was employed, to avoid a double Ugi reaction (Fig. 6).

For the 1,5-disubstituted tetrazoles **21**, the substituent in 1-position of the tetrazole moiety was varied, too. In Ugi reactions, variation of the substituent in 1-position of the resulting tetrazole can be realized by employing variably substituted isonitriles. For this purpose, apart from 1,1,3,3-tetramethylbutyl isonitrile (**33**), cyclohexyl isocyanide (**34**) and benzyl isocyanide (**35**) were chosen (Fig. 7).

2.1.1. Synthesis of the amines

The synthesis of not commercially available amines **25**–**28** could easily be accomplished following literature procedures or using well-established reactions. Thus, **26** was synthesized according to a two-step procedure of van der Bent et al.[43] starting from benzophenone (71% yield). Likewise, 4,4-diphenylbut-3-en-1-ylamine (**27**) was assembled starting from cyclopropyldiphenylcarbinole according to reported procedures[43,24,44] (50% over three steps). Subsequent reduction of the double bond with triethylsilane in CF_3COOH yielded amine **25**[29,45] in 69% yield. The reaction of carbazole with 2-(3-bromopropyl)isoindole-1,3-dione with subsequent hydrazinolyses gave 3-carbazol-9-ylpropylamine (**28**)[46] in 91% yield.

2.1.2. Synthesis of the 1,5-disubstituted tetrazoles

The Ugi reaction was performed by simply mixing equimolar amounts of paraformaldehyde (**36**), the respective amine **22**–**32**, the isonitrile **33**–**35** and TMS-azide (**37**) in MeOH and subjecting the mixture to microwave irradiation for 1.5 h at 80 °C (Scheme 1).[‡]

When employing sterically less hindered primary amines, the amount of the amine was doubled to suppress the formation of byproducts, such as **38** (Fig. 8), resulting from a repetitive Ugi reaction. Thus, regardless the nature of the amine or the isocyanide, the desired 1,5-disubstituted tetrazoles of the general structure **21** were isolated with good to excellent yields ranging from 56% to 99%. The results of the $TMSN_3$-modified Ugi reaction are summarized in Fig. 9.

As mentioned above, to avoid a double Ugi reaction, the mono-Boc-protected diamines **30** and **31** have been employed. Therefore, for the syntheses of **62** and **63**·2HCl, subsequent to the Ugi reaction, the Boc-groups had to be cleaved off. For compounds **59** and **60**, both *N*-Boc- and tmb-protected, selective Boc-deprotection could be achieved at room temperature, using hydrochloric acid in ethanol. Thus, the amines **62** and **63**·2HCl were obtained in reasonable to excellent yields (Scheme 2).

2.1.3. Synthesis of the 5-monosubstituted tetrazoles

From the tmb-protected tetrazole derivatives **39**, **42**, **45**, **48**, **51**, **54**, **57**, **58**, and **61** the protective group at the tetrazole moiety was cleaved off to obtain the amino acid analogs of the general structure **20**. As described by Dömling et al., this is easily accomplished in acidic medium.[42] For the deprotection of tetrazoles mostly elevated temperatures were needed, therefore the 1,5-disubstituted

[‡] Similar results, however, could be obtained by stirring the reaction mixture under reflux conditions.

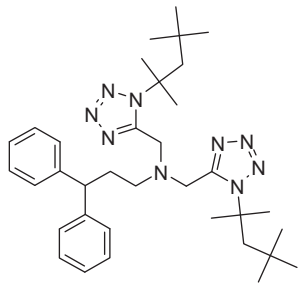


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Figure 8. Product of a repetitive Ugi reaction.

tetrazole derivatives **39**, **42**, **45**, **48**, **51**, **54**, **57**, **58**, and **61** were refluxed in an excess of HCl_{EtOH}. This way, the amino acid analogs **64**–**72** were obtained in good to excellent yields ranging from 65% to 99% (cf. Fig. 10). By the same procedure, starting from **59** and **60**, combined cleavage of the Boc- and tmb-groups could be accomplished, resulting in **73**·2HCl[49] and **74**·2HCl in 91% and 31% yield, respectively (Fig. 10).

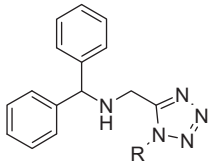
For the synthesis of the unsubstituted tetrazole amino acid **19**·HCl[50,51] the diphenylmethyl- and tetramethylbutyl protected tetrazole derivative **39** was employed. Both protecting groups could easily be removed by refluxing **39** in a mixture of triethylsilane and TFA. Subsequent purification by ion exchange chromatography and treatment with an excess of HCl gave the hydrochloride salt **19**·HCl in 70% yield (Scheme 3).

2.2. Pharmacology

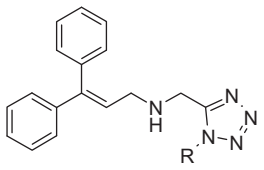
All 1,5-disubstituted and 5-monosubstituted tetrazole derivatives were characterized regarding their inhibitory potency and subtype selectivity at the four GABA transporter subtypes mGAT1–mGAT4. This was accomplished using a uniform [^{3}H]GABA uptake assay on HEK cell lines, each stably expressing one of the four GATs.[52] The potency of the tested compounds is given as pIC_{50} value. For test compounds of low potency, unable to reduce the specific [^{3}H]GABA uptake to a value below 50% at a specified concentration (100 μM, 1 mM, 10 mM), no pIC_{50} values were determined. For these compounds, the percentage of the remaining specific [^{3}H]GABA uptake at the defined concentration is given.

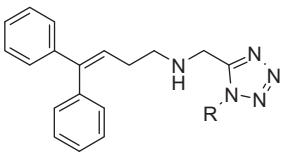
As reference compounds for the unsubstituted glycine analog **19**·HCl, the amino acid glycine (**18**) and its homolog, β-alanine (**12**), were chosen. The inhibitory effect of glycine (**18**) on GABA-uptake is only moderate (Table 1, entry 1). By contrast, β-alanine (**12**), shows good activity at mGAT3 and mGAT4, with remarkable selectivity especially towards mGAT1 (Table 1, entry 2).[53]

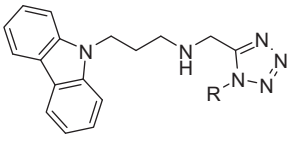
Compounds NNC 05-2090 (**7**) and (*S*)-SNAP-5114 (**10**) served as references for inhibitors with pronounced activity at mGAT2 or



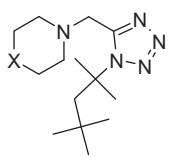
39[a]	R = tmb	78%	**42**[b]	R = tmb	94%	**45**[b]	R = tmb	92%	**48**[b]	R = tmb	99%
40[a]	R = Cyclohexyl	70%	**43**[b]	R = Cyclohexyl	92%	**46**[b]	R = Cyclohexyl	72%	**49**[b]	R = Cyclohexyl	76%
41[b]	R = Bn	95%	**44**[b]	R = Bn	86%	**47**[b]	R = Bn	85%	**50**[b]	R = Bn	70%

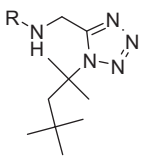






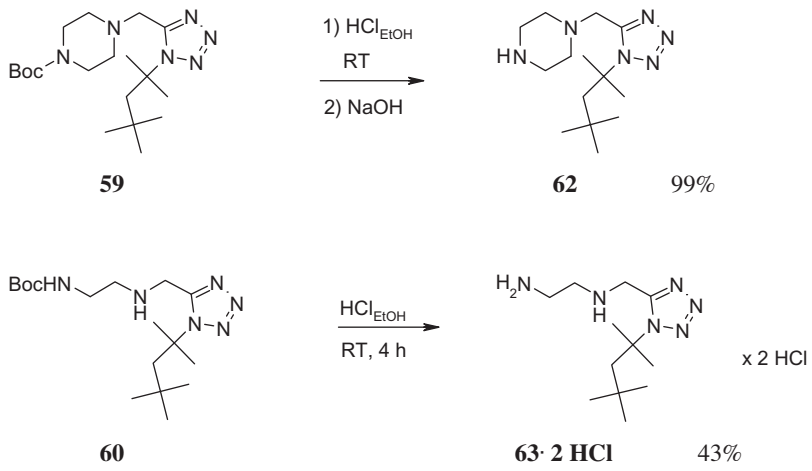
51[b]	R = tmb	92%	**54**[b]	R = tmb	82%	**57**[a] R = tmb 56%
52[b]	R = Cyclohexyl	76%	**55**[b]	R = Cyclohexyl	98%	
53[b]	R = Bn	59%	**56**[b]	R = Bn	73%	





58[a]	X = O	79%	**60**[a]	R^1 = $BocHNCH_2CH_2$	80%
59[a]	X = NHBoc	82%	**61**[a]	R^1= $CH_3CH_2CH_2CH_2$	63%

Figure 9. Results of the $TMSN_3$-modified Ugi reaction (a: 1 equiv amine employed; b: 2 equiv amine employed; tmb: 1,1,3,3-tetramethylbutyl).



Scheme 2. Syntheses of **62** and **63**·2HCl.

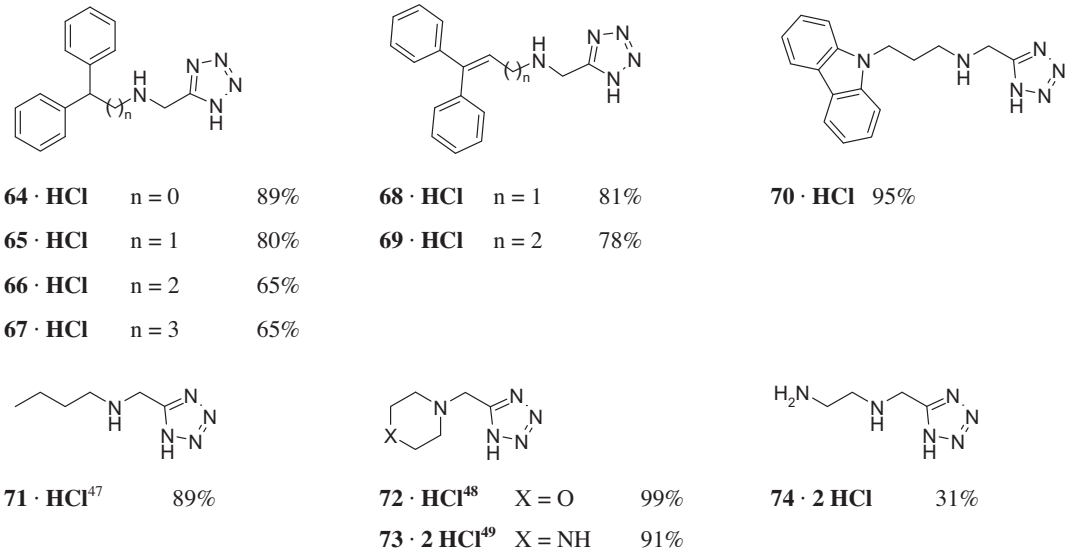
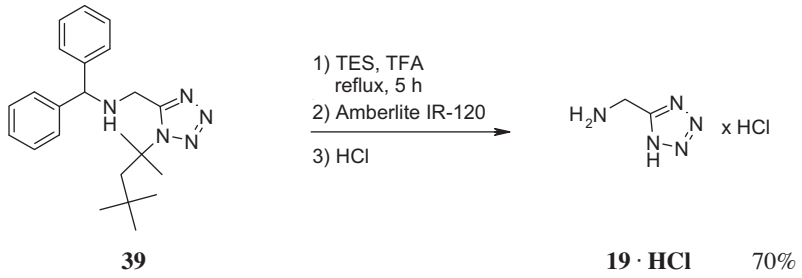


Figure 10. Results of the acidic cleavage of the protecting groups. (See above-mentioned references for further information)



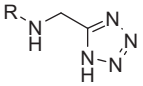
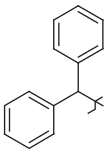
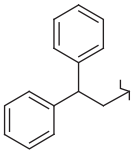
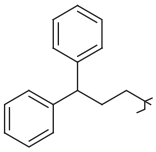
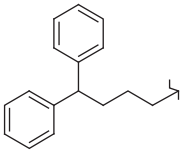
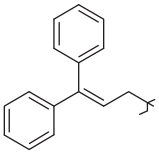
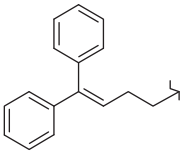
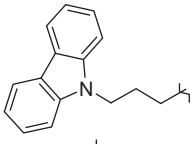
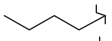
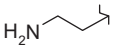
Scheme 3. Synthesis of **19**·HCl.

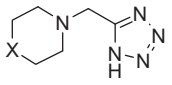
mGAT3 and mGAT4, respectively. To allow a better comparability of the test results obtained for the newly synthesized compounds presented in Tables 2 and 3 with those of the reference compounds in Table 1, the reference compounds were evaluated for their inhibitory potencies at the individual GABA transporters employing the test system of our group, too. For **7** and **10** published results are given in parentheses (Table 1). NNC 05-2090 (**7**) has been characterized as mGAT2 selective inhibitor by Thomsen et al.[28] This selectivity, however, could not be reproduced applying the test system established in our group. Still, NNC 05-2090 (**7**) has been found to be a potent inhibitor of GABA uptake, albeit with no reasonable selectivity for one of the transporter subtypes (Table 1, entry 3). (*S*)-SNAP-5114 (**10**), originally published by Dhar et al.,[31] shows high activity at mGAT3 (rGAT-2) and, especially, at mGAT4 (and hGAT-3, respectively), with a good subtype selectivity (>57:1) for mGAT4, as compared to mGAT1 and mGAT2 (Table 1, entry 4).[§]

In Table 2 the results of the biological evaluation of the amino acid analogs are summarized. The tetrazole analog of glycine,

[§] For compounds reducing [^{3}H]GABA uptake by not more than 50% at 1 mM a $pIC_{50} \leqslant 3$ and at 100 μM a $pIC_{50} \leqslant 4$ is assumed.

Table 2
Uptake inhibition at mGAT1–mGAT4

Entry			mGAT1 (%)	mGAT2 (%)	mGAT3 (%)	mGAT4 (%)
	No.	R				
1	**19**·HCl	H	85.1[a]	59.4[a]	66.5[a]	78.9[a]
2	**64**·HCl		69.7	94.2	94.2	85.4
3	**65**·HCl		48.2	85.8	63.5	71.2
4	**66**·HCl		100	53.2	72.6	100
5	**67**·HCl		82.0	89.3	75.1	87.4
6	**68**·HCl		80.5	82.0	46.2	79.6
7	**69**·HCl		86.1	76.9	73.8	101
8	**70**·HCl		86.3	81.6	83.6	78.7
9	**71**·HCl		105	65.3	74.0	71.5
10	**74**·2HCl		81.7[a]	80.0[a]	86.3[a]	60.7[a]

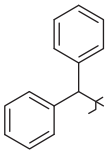
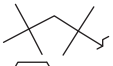
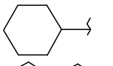
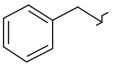
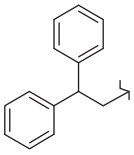
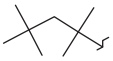
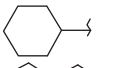
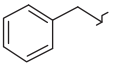
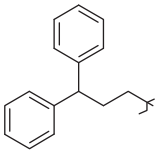
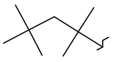
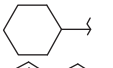
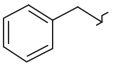
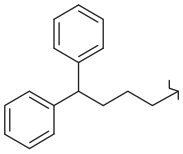
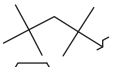
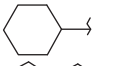
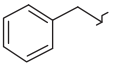
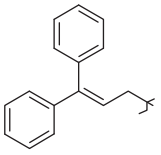
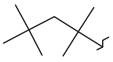
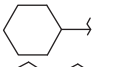
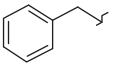
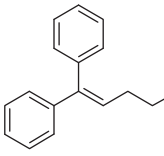
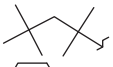
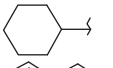
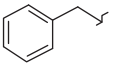
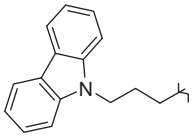
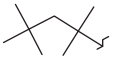
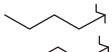
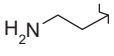
Entry			mGAT1	mGAT2	mGAT3	mGAT4
	No.	X				
11	**73**·2HCl	NH	90.0[a]	75.4[a]	102[a]	101[a]
12	**72**·HCl	O	90.6[a]	88.0[a]	87.8[a]	90.8[a]

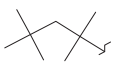
Percentages represent specific binding remaining in presence of 100 μM inhibitor.
[a] 1 mM inhibitor.

19·HCl, gave results comparable to the amino acid **18** itself. Thus, at mGAT2 and mGAT3 moderate inhibition of GABA-uptake was measurable (Table 2, entry 1). The larger size of the tetrazole compared to the carboxylic acid apparently did not result in a higher activity, for example comparable to β-alanine (**12**). When it comes to the lipophilic derivatives of **19**, contrary to expectations, no enhanced activity was observed. Regardless the length of the spacer, no beneficiary effect of the bis-aromatic substituents as compared to a pure alkyl-residue was found (Table 2, entries 2–7, and 9). Neither did substitution with a carbazolylpropyl residue, which contributes to the activity of NNC 05-2090 (**10**), result in an increase in potency (Table 2, entry 8).

As far as the cyclic amino acid analogs **73**·2HCl and **72**·HCl are concerned, both showed only negligible activity at the four GABA

Table 3
Uptake inhibition at mGAT1–mGAT4

Entry	No.	R^1	R^2	mGAT1 (%)	mGAT2	mGAT3	mGAT4
1	**39**			85.6	81.4%	**4.22 ± 0.11**	56.9%
2	**40**			54.8	76.3%	**4.61 ± 0.09**	**4.54 ± 0.05**
3	**41**			81.1	84.5%	**4.79 ± 0.13**	57.2%
4	**42**			83.3	63.5%	53.8%	**4.17 ± 0.10**
5	**43**			59.1	75.9%	**4.37 ± 0.10**	**4.35 ± 0.09**
6	**44**			72.4	67.1%	**4.35 ± 0.09**	52.3%
7	**45**			70.7	77.1%	**5.09 ± 0.13**	**4.53 ± 0.04**
8	**46**			54.6	74.8%	**4.44 ± 0.02**	**4.46 ± 0.09**
9	**47**			73.9	61.6%	51.7%	**4.20 ± 0.15**
10	**48**			63.8	**4.78 ± 0.13**	**4.89 ± 0.02**	**4.53**[b]
11	**49**			70.6	**4.66 ± 0.16**	**4.76 ± 0.05**	43.2%
12	**50**			68.1	**4.81 ± 0.25**	**4.39**[c]	49.6%
13	**51**			65.7	**4.99 ± 0.04**	**4.64**[b]	**4.58**[b]
14	**52**			62.9	**4.61 ± 0.08**	**4.74**[b]	**4.29**[b]
15	**53**			57.3	**4.61 ± 0.72**	**4.46**[b]	**4.38**[b]
16	**54**			75.4	54.9%	**4.91 ± 0.17**	**4.74 ± 0.13**
17	**55**			64.5	69.5%	**4.67 ± 0.10**	58.2%
18	**56**			69.4	84.1%	53.5%	53.1%
19	**57**			75.1	72.6%	68.8%	59.7%
20	**61**			83.3	60.3%	49.0%	**4.31**[b]
21	**63**·2HCl			133[a]	142%[a]	71.1%[a]	92.5%[a]

Entry	No.	X	R	mGAT1	mGAT2	mGAT3	mGAT4
22	**58**	O		73.4	99.0%	63.3%	90.5%
23	**62**	NHBoc		90.2	106.0%	81.4%	78.4%

If not stated otherwise, the results are given as pIC_{50} ± SEM of three independent experiments, each performed in triplicate. Percentages represent specific binding remaining in presence of 100 μM inhibitor.

[a] 1 mM inhibitor.

[b] One experiment, performed in triplicate.

[c] Mean of two independent experiments, each performed in triplicate.

transport proteins (Table 2, entries 11–12). The diamine **74**·2HCl, too, was not able to considerably reduce GABA uptake (Table 2, entry 10).

A totally different pharmacological profile was obtained for the 1,5-disubstituted tetrazole-derivatives. Although, just like the 5-monosubstituted tetrazoles, all 1,5-disubstituted tetrazole derivatives showed only low to marginal inhibition of GABA uptake at mGAT1, considerable inhibition of the non-GAT1 transporters was observed for some of the test compounds (Table 3). Thus, the aminomethyltetrazole derivatives substituted with a diphenylmethyl group at the amine nitrogen, **39**–**41**, were found to be moderately active inhibitors of mGAT3 and mGAT4, with a slightly higher potency at mGAT3 (Table 3, entries 1–3). Compounds **42**–**44**, bearing a diphenylethyl residue at the amine nitrogen, however, proved to be somewhat less potent as compared to **39**–**41** (Table 3, entries 4–6). When it comes to the diphenylpropyl-derivatives, **45**–**47**, pronounced differences in activity were observed between the in 1-position differently substituted tetrazole derivatives (Table 3, entries 7–9). Here, the tetramethylbutyl derivative **45** displayed the highest inhibitory potency at mGAT3, combined with a reasonable subtype selectivity for this transporter subtype (pIC_{50} 5.09, selectivity mGAT3/mGAT1 = 12:1, mGAT3/mGAT4 = 4:1; Table 3, entry 7). Although the potency of **45** at mGAT4 is approximately 20 times lower than the potency of (*S*)-SNAP 5114 (**10**), its inhibitory effect on GABA-uptake at mGAT3 is comparable (Table 1, entry 4).

For **48**, **49** and **50**, which are substituted with a diphenylbutyl residue at the amine nitrogen, in addition to a moderate to good activity at the transporter subtypes mGAT3 and mGAT4, significant inhibition of GABA uptake at mGAT2 was observed (Table 3, entries 10–12). With the tetramethylbutyl substituted tetrazole derivative **48**, a compound with good activity at the non-GAT1 transporter subtypes was identified (pIC_{50} mGAT2: 4.78, pIC_{50} mGAT3: 4.89, pIC_{50} mGAT4: 4.53; Table 3 entry 10). Furthermore, good activity at mGAT2 (pIC_{50} 4.81) combined with a moderate selectivity towards mGAT1, mGAT3, and mGAT4 (>3:1) was observed for the benzyl derivative **50** (Table 3, entry 12).

As far as the unsaturated **51**–**53** and **54**–**56** are concerned, there are distinct differences in the inhibitory potency compared to their saturated analogs **45**–**47** and **48**–**50**. Among the diphenylpropenyl derivatives **51**–**53**, especially compound **51** displayed remarkable uptake inhibition at mGAT2 (pIC_{50} 4.99; Table 3, entry 13), which is comparable to the activity of NNC 05-2090 (**7**, pIC_{50} 5.09; Table 1, entry 3). But, in contrast to the results we obtained for NNC 05-2090 (**7**), **51** showed good subtype selectivity as compared to mGAT1 (selectivity mGAT2/mGAT1 $\geqslant$ 10:1). For **52** and **53** moderate inhibition of non-GAT1 transport proteins was found, but without any preference for one of the three transporter subtypes (Table 3, entries 14–15).

When it comes to the aminomethyltetrazole derivates substituted with a diphenylbutenyl residue, the activity at mGAT2 vanishes. Compound **56**, bearing a benzyl group in 1-position of the tetrazole, has only negligible activity at all four transporter subtypes (Table 3, entry 18). By contrast, for **54** and **55** moderate to good uptake inhibition at mGAT3 was observed (Table 3, entries 16–17). While **55** was found to be moderately active only at mGAT3 (pIC_{50} 4.67), **54** displayed good activity at mGAT3 and mGAT4 (pIC_{50} mGAT3: 4.91, pIC_{50} mGAT4: 4.74).

Although it is substituted with a carbazolylpropyl residue, which can also be found in NNC 05-2090 (**7**), scarcely any uptake inhibition could be detected for **57** (Table 3, entry 19). Likewise, **61**, with a butyl group attached to the amine nitrogen did not display particular activity at mGAT1–mGAT4 (Table 3, entry 20). Also compounds **58**, **62** and **63**·2HCl that completely lack a lipophilic side chain but display additional rings or amino groups instead, were found to be more or less inactive at all four GABA transporter subtypes (Table 3, entries 21–23). These results suggest that for the activity of 1,5-disubstituted aminomethyltetrazoles of the general structure **21**, a lipophilic side chain containing a bis-aromatic substructure is necessary.

3. Conclusion

In summary, 1,5-disubstituted and 5-monosubstituted tetrazole derivatives derived from glycine (**18**) have been synthesized. All compounds have been evaluated regarding their inhibitory potency and subtype selectivity at the four murine GABA transporter subtypes mGAT1–mGAT4. The results for the N-substituted scaffold type **20** were far from satisfying. None of the amino acid analogs was capable of inhibiting the [^{3}H]GABA uptake by more than approximately 50% at the highest concentration employed (1 mM and 100 μM, respectively). But, interestingly, the 1,5-disubstituted tetrazole derivatives belonging to structural type **21** displayed by far higher activity, especially at the GABA transport proteins mGAT2–mGAT4. Thereby a moderate to good selectivity against the GABA transporter mGAT1 was observed. With **45**, a reasonable potent and selective inhibitor of mGAT3 was found. Additionally **50** and **51** were identified as potent inhibitors of mGAT2. This is especially relevant, as up to date only few potent inhibitors of mGAT2 that do not affect mGAT1 are known. Although no general dependency from chain length or substituent in 1-position of the tetrazole moiety could be determined, these compounds might represent a good starting point for the development of a new class of non-GAT1 selective GABA-uptake inhibitors.

4. Experimental

4.1. Chemistry

4.1.1. General experimental

Anhydrous reactions were carried out in vacuum dried glassware under argon atmosphere. Purchased reagents were used without further purification. THF was freshly distilled from sodium metal/benzophenone ketyl, and CH_3OH from Mg prior to use. Other solvents for extraction, recrystallization and column chromatography were distilled before use. Reactions under microwave irradiation were performed in sealed glass vials in a Biotage Initiator laboratory microwave. Column chromatography (CC) was performed as flash-column chromatography according to Still[54] on silica gel 60 (0.035–0.070 mm, Acros and Merck). TLC plates were made from silica gel 60 F254 (Merck). Detection was effected in UV or by staining the TLC plates with 5% $(NH_4)_6Mo_7O_{24} \times 4H_2O$ and 0.2% $Ce(SO_4)_2 \times 4H_2O$ in 5% H_2SO_4. MPLC purification was performed using a Sepacore® column B-685 (26 × 230 mm) filled with silica gel RP 18 as stationary phase. Solvents for MPLC were of p.a. quality and degassed prior to use. Melting points were determined on a Büchi 510 melting point apparatus or a Barnstead Electrothermal melting pot apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer FT-Infrared Spectrometer Paragon 1000. Solids were measured as KBr pellets, oily samples as film on NaCl plates. ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a JEOL JNMR-ECP 400 or a JEOL JNMR-ECP 500 using TMS as internal standard. Samples without TMS were adjusted to the signal of the partially deuterated solvent. For samples in D_2O, dioxane was added as internal standard. NMR spectra were analyzed with the NMR software MestReNova, Version 5.1.0-2940 (Mestrelab Research S.L.). Mass spectra were measured on a Mass Spectrometer 5989 A with 59980 B particle beam LC/MS interface (Hewlett Packard) or an Applied Biosystems LC–MS/MS-Mass Spectrometer API 2000. High resolution mass spectrometry (HRMS) was accomplished with a LTQ FT (ThermoFinnigan) or a

JMS GCmate II (Jeol). Micoranalytical data for carbon, hydrogen, and nitrogen were determined on an Elementar Vario Micro Cube or an Elementar Vario EL analyzer.

4.1.2. General procedures

4.1.2.1. General procedure 1 (GP1). To a solution of paraformaldehyde (1.0 mmol) in MeOH (10.0 mL) the respective amine (1.0 or 2.0 mmol), the isocyanide (1.0 mmol) and $TMSN_3$ (1.0 mmol) were added. The resulting solution was stirred at 80 °C in the microwave for 1.5 h. After addition of phosphate buffer solution (1 M, pH 7) and water (approx. 10 mL each) the crude product was extracted with CH_2Cl_2 (5 × approx. 20 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was removed in vacuo. Further purification of the tetrazole product was performed as specified.

4.1.2.2. General procedure for the acidic cleavage of the tmb-group (GP2). The protected tetrazole derivative was treated with an excess (approx. 15–19 equiv) of HCl_{EtOH} (~1.25 M) and was refluxed overnight (8–13 h). The solvent was removed in vacuo and the oily residue was treated with HCl_{ether}. In doing so, the product precipitated as a colorless solid. After the precipitation was complete, the product was filtered off and washed with Et_2O, if necessary.

4.1.3. Individual procedures

4.1.3.1. Benzhydryl-(1-benzyl-1*H*-tetrazol-5-ylmethyl)amine (41). According to **GP1** starting from paraformaldehyde (57.1 mg, 1.90 mmol), benzhydrylamine (349.0 mg, 1.904 mmol, 328.0 μL), benzyl isocyanide (223.2 mg, 1.905 mmol, 232.0 μL) and TMS azide (115.6 mg, 1.003 mmol, 132.0 μL). Purification by CC (*n*-pentane/EtOAc = 1:1) yielded a yellowish oil that was diluted in mobile phase (approx. 2 mL). After ultrasonication of the solution for approx. 30 s the product precipitated as colorless solid and was isolated by filtration.

Compound ***41***. Colorless solid (337.7 mg, 95%); mp 132 °C. R_f = 0.63 (*n*-pentane/EtOAc). IR: 3026, 2817, 1964, 1825, 1583, 1532, 1492, 1456, 1111, 752, 707, 713, 700 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 2.11 (s_{br}, 1H, NH), 3.90 (s, 2H, CH_2NH), 4.80 (s, 1H, CH(C_6H_5)$_2$), 5.53 (s, 2H, C$H_2C_6H_5$), 7.01–7.09 (m, 2H, $H_{ortho,Bn}$), 7.21–7.38 (m, 13H, H_{ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 40.43, 51.14, 66.89, 127.45, 127.73, 127.77, 128.94, 128.99, 129.31, 133.67, 142.62, 153.48 ppm. M ($C_{22}H_{21}N_5$) = 355.45. MS (CI, CH_5^+) *m*/*z* (%): 356 (25, $[M+H]^+$), 182 (20), 167 (100). HRMS (ESI^+): $[M+H]^+$ calcd for $C_{22}H_{22}N_5$, 356.1870. Found: 356.1862.

4.1.3.2. (1-Cyclohexyl-1*H*-tetrazol-5-ylmethyl)-(2,2-diphenylethyl)amine (43). According to **GP1** starting from paraformaldehyde (59.0 mg, 1.97 mmol), 2,2-diphenylethylamine (754.0 mg, 3.822 mmol), cyclohexyl isocyanide (208.1 mg, 1.906 mmol, 237.0 μL) and $TMSN_3$ (219.0 mg, 1.901 mmol, 250.0 μL). Purification by CC (*n*-pentane/EtOAc = 4:6) and subsequent recrystallization from CH_2Cl_2/*n*-pentane (3:7) yielded **43**.

Compound ***43***. Colorless crystals (633.1 mg, 92%); mp 109 °C. R_f = 0.37 (*n*-pentane/EtOAc = 4:6). IR: 3300, 3025, 2934, 2861, 1946, 1599, 1494, 1449, 1438, 1105, 742, 696 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 1.22–1.41 (m, 3H, CHCH_2CH_2CH_2, CHCH_2 CH_2C$H_{2,ax}$), 1.56 (s_{br}, 1H, NH), 1.69–1.77 (m, 1H, CHCH_2CH_2C$H_{2,eq}$), 1.85–2.01 (m, 6H, CHCH_2CH_2CH_2, CHC$H_{2,ax}$CH_2CH_2, CHCH_2 C$H_{2,eq}$CH_2), 3.26 (d, *J* = 7.6 Hz, 2H, CH_2CH), 4.09 (s, 2H, NCH_2C), 4.14 (t, *J* = 7.6 Hz, 1H, CH_2CH), 4.31 (tt, *J* = 10.9/4.3 Hz, 1H, CHCH$_2$CH$_2$CH$_2$), 7.18–7.23 (m, 6H, $H_{ar,ortho}$, $H_{ar,para}$), 7.25–7.31 (m, 4H, $H_{ar,meta}$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ = 25.03, 25.46, 32.95, 42.40, 51.32, 54.02, 58.12, 126.93, 128.13, 128.90, 142.54, 152.27 ppm. M ($C_{22}H_{27}N_5$) = 361.49. MS (CI, CH_5^+) *m*/*z* (%): 362 (100, $[M+H]^+$), 210 (32), 194 (48), 112 (22). HRMS (ESI^+): $[M+H]^+$ calcd for $C_{22}H_{28}N_5$, 362.2340; found: 362.2340. Anal. calcd for C 73.10, H 7.53, N 19.37. Found: C 72.68, H 7.37, N 19.29.

4.1.3.3. (3,3-Diphenylpropyl)-[1-(1,1,3,3-tetramethylbutyl)-1*H*-tetrazol-5-ylmethyl]amine (45) and (3,3-diphenylpropyl)-bis-[1-(1,1,3,3-tetramethylbutyl)-1*H*-tetrazol-5-ylmethyl]amine (38). *Method A*: According to **GP1** starting from paraformaldehyde (58.6 mg, 1.95 mmol), 3,3-diphenylpropylamine (805.6 mg, 3.812 mmol, 2 equiv), 1,1,3,3-tetramethylbutyl isocyanide (265.2 mg, 1.905 mmol, 334.0 μL) and $TMSN_3$ (219.0 mg, 1.901 mmol, 250.0 μL). After purification by CC (*n*-pentane/EtOAc = 4:6) **45** was isolated as sole product.

Compound ***45***. Colorless oil (709.3 mg, 92%). R_f = 0.40 (*n*-pentane/EtOAc = 4:6). IR: 3324, 3026, 2952, 2871, 1808, 1599, 1583, 1493, 1471, 1451, 1233, 1114, 751, 702 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 0.76 (s, 9H, $(CH_3)_3$), 1.76 (s, 6H, NC$(CH_3)_2$), 1.78 (s_{br}, 1H, NH), 1.96 (s, 2H, CH_2C$(CH_3)_3$), 2.28 (q, *J* = 7.5 Hz, 2H, CH_2CH), 2.66 (t, *J* = 7.2 Hz, 2H, CH$_2$CH_2NH), 4.05 (t, *J* = 7.9 Hz, 1H, CH$_2$CH), 4.08 (s, 2H, NCH$_2$C), 7.14–7.19 (m, 2H, $H_{ar,para}$), 7.21–7.30 (m, 8H, $H_{ar,meta}$, $H_{ar,ortho}$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ = 30.16, 30.85, 31.81, 35.95, 45.16, 48.43, 49.02, 53.42, 64.79, 126.47, 127.96, 128.72, 144.70, 153.52 ppm. M ($C_{25}H_{35}N_5$) = 405.59. MS (CI, CH_5^+) *m*/*z* (%): 406 (8, $[M+H]^+$), 294 (100), 210 (10). HRMS (EI, 70 eV): M^+ calcd for $C_{25}H_{35}N_5$, 405.28925; found: 405.29145.

Method B: When the synthesis of **45** was conducted according to **GP1** starting from paraformaldehyde (60.6 mg, 2.02 mmol), 3,3-diphenylpropylamine (408.4 mg, 1.933 mmol, 1 equiv), 1,1,3,3-tetramethylbutyl isocyanide (265.2 mg, 1.905 mmol, 334.0 μL) and $TMSN_3$ (219.0 mg, 1.901 mmol, 250.0 μL), besides approx. 86% of the Ugi product **45** the byproduct **38** occurred. **38** was isolated by CC (*n*-pentane/EtOAc = 1:1).

Compound ***38***. Colorless solid. (49.4 mg, 9%); mp: 116–117 °C. R_f = 0.71 (*n*-pentane/EtOAc = 1:1). IR: 3059, 3028, 2954, 2870, 1952, 1881, 1598, 1450, 1375, 1229, 1114, 1093, 771, 708 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 0.61 (s, 18H, $(CH_3)_3$), 1.63 (s, 12H, NC$(CH_3)_2$), 1.78 (s, 4H, CH_2C$(CH_3)_3$), 2.21–2.27 (m, 2H, CH_2CH(C_6H_5)$_2$), 2.85–2.90 (m, 2H, NCH_2CH$_2$CH), 3.97 (t, *J* = 7.8 Hz, 1H, CH(C_6H_5)$_2$), 4.34 (s, 4H, NCH$_2$C), 7.05–7.11 (m, 2H, $H_{ar,para}$), 7.14–7.20 (m, 8H, $H_{ar,meta}$, $H_{ar,ortho}$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 30.19, 30.81, 31.76, 33.21, 47.97, 48.69, 53.17, 53.46, 64.90, 126.57, 127.95, 128.78, 144.43, 152.20 ppm. M ($C_{35}H_{53}N_9$) = 613.90. MS (CI, CH_5^+) *m*/*z* (%): 600 (25, $[M+H]^+$), 488 (100), 376 (52), 113 (29). HRMS (EI, 70 eV): M^+ calcd for $C_{35}H_{53}N_9$, 599.4424; found: 599.4457.

4.1.3.4. (3,3-Diphenylpropyl)-(1*H*-tetrazol-5-ylmethyl)amine hydrochloride (66·HCl). Deprotection of **45** (142.0 mg, 0.3501 mmol) according to **GP2** using HCl_{EtOH} (~1.25 M, 10 mmol, 8.0 mL, 29 equiv). Reaction time: 8 h. After removal of the excess solvent and addition of HCl in Et_2O the formed precipitate was washed with EtOAc.

Compound ***66***. HCl. Colorless crystals (75.1 mg, 65%); mp 168 °C. IR: 3440, 3059, 2957, 2758, 1959, 1869, 1598, 1493, 1493, 1450, 1020, 749, 704 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ = 2.47–2.56 (m, 2H, CH_2CH), 3.08–3.15 (m, 2H, CH_2CH$_2$CH), 4.07 (t, *J* = 7.9 Hz, 1H, CH(C_6H_5)$_2$), 4.60 (s, 2H, CCH_2NH), 7.17–7.23 (m, 2H, $H_{ar,para}$), 7.27–7.33 (m, 8H, $H_{ar,ortho}$, $H_{ar,meta}$) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ = 32.67, 41.93, 48.17, 49.84, 127.88, 128.73, 129.86, 144.68, 155.88 ppm. M ($C_{17}H_{19}N_5$ × HCl) = 329.83. MS (CI, CH_5^+) *m*/*z* (%): 294 (24, $[M+H]^+$), 212 (91), 145 (100), 103 (81). HRMS (ESI^+): $[M+H]^+$ calcd for $C_{17}H_{20}N_5$, 294.1713; found: 294.1713.

4.1.3.5. (4,4-Diphenylbutyl)-[1-(1,1,3,3-tetramethylbutyl)-1*H*-tetrazol-5-ylmethyl]amine (48). According to **GP1** starting from paraformaldehyde (17.5 mg, 0.583 mmol), 4,4-diphenylbutylamine (225.1 mg, 0.9989 mmol), 1,1,3,3-tetramethylbutyl

isonitrile (69.9 mg, 0.502 mmol, 88.0 μL) and trimethylsilyl azide (57.8 mg, 0.502 mmol, 66.0 μL). Purification by CC (*n*-pentane/EtOAc = 1:1) gave a colorless oil.

Compound ***48***. Colorless oil (202.9 mg, 96%). R_f = 0.61 (*n*-pentane/EtOAc = 35:65). IR: 3329, 3025, 2950, 1948, 1806, 1673, 1599, 1493, 1451, 1231, 1114, 749, 702 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 0.76 (s, 9H, $(CH_3)_3$), 1.46–1.55 (m, 2H, $CH_2CH_2CH_2$), 1.78 (s, 6H, $NC(CH_3)_2$), 1.96 (s, 2H, $CH_2C(CH_3)_3$), 2.07–2.13 (m, 2H, $CH_2CH(C_6H_5)_2$), 2.34 (s_{br}, 1H, NH), 2.74 (t, J = 7.1 Hz, 2H, $HNCH_2CH_2$), 3.89 (t, J = 7.8 Hz, 1H, $CH(C_6H_5)_2$), 4.13 (s, 2H, CCH_2NH), 7.14–7.18 (m, 2H, $H_{ar,para}$), 7.20–7.24 (m, 4H, $H_{ar,ortho}$), 7.24–7.29 (m, 4H, $H_{ar,meta}$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ = 28.18, 29.91, 30.64, 31.60, 33.15, 44.71, 49.50, 51.14, 53.20, 64.67, 126.16, 127.78, 128.44, 144.79, 153.05 ppm. M ($C_{26}H_{37}N_5$) = 419.62. MS (CI, CH_5^+) m/z (%): 420 (9, $[M+H]^+$), 308 (100). HRMS (EI, 70 eV): M^+ calcd for $C_{26}H_{37}N_5$, 419.30490; found: 419.30567.

4.1.3.6. (1-Benzyl-1*H*-tetrazol-5-ylmethyl)-(4,4-diphenylbutyl) amine (50). According to **GP1** starting from paraformaldehyde (15.0 mg, 0.500 mmol), 4,4-diphenylbutylamine (205.4 mg, 0.9115 mmol), benzyl isocyanide (58.7 mg, 0.501 mmol, 61.0 μL) and TMS azide (57.8 mg, 0.502 mmol, 66.0 μL). Purification by CC (*n*-pentane/EtOAc = 1:1).

Compound ***50***. Pale yellow oil (139.2 mg, 70%). R_f = 0.16 (*n*-pentane/EtOAc = 1:1). IR: 3331, 3060, 3026, 2934, 2859, 1952, 1812, 1599, 1494, 1452, 1360, 1115, 725, 702 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 1.36–1.43 (m, 2H, $CH_2CH_2CH_2$), 1.64 (s_{br}, 1H, NH), 1.99–2.06 (m, 2H, CH_2CH), 2.56 (t, J = 7.1 Hz, 2H, $HNCH_2CH_2$), 3.84 (t, J = 7.8 Hz, 1H, $CH(C_6H_5)_2$), 3.90 (s, 2H, CCH_2NH), 5.62 (s, 2H, $CH_2C_6H_5$), 7.14–7.22 (m, 8H, H_{ar}), 7.24–7.34 (m, 7H, H_{ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.21, 33.07, 42.22, 49.33, 51.05, 51.11, 126.21, 127.70, 127.74, 128.48, 128.84, 129.11, 133.52, 144.72, 153.15 ppm. M ($C_{25}H_{27}N_5$) = 397.53. MS (CI, CH_5^+) m/z (%): 398 (100, $[M+H]^+$), 238 (15). HRMS (EI, 70 eV): M^+ calcd for $C_{25}H_{27}N_5$, 397.22665; found: 397.22747.

4.1.3.7. (4,4-Diphenylbutyl)-(1*H*-tetrazol-5-ylmethyl)amine hydrochloride (67·HCl). According to **GP2** starting from **48** (190.2 mg, 0.4533 mmol) in HCl_{EtOH} (~1.25 M, 8.8 mmol, 7.0 mL, 19 equiv). The reaction mixture was refluxed for 8 h.

Compound ***67***. HCl. Colorless solid (101.4 mg, 65%); mp 154–156 °C dec IR: 3081, 3024, 2954, 2767, 2414, 1954, 1718, 1597, 1492, 1450, 1023, 745, 703 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ = 1.65–1.77 (m, 2H, $CCH_2CH_2CH_2N$), 2.12–2.23 (m, 2H, $CCH_2CH_2CH_2N$), 3.14–3.25 (m, 2H, $CCH_2CH_2CH_2N$), 3.97 (t, J = 7.9 Hz, 1H, $CH(C_6H_5)_2$), 4.57 (s, 2H, CCH_2NH), 7.11–7.21 (m, 2H, $H_{ar,para}$), 7.22–7.33 (m, 8H, $H_{ar,meta}$, $H_{ar,ortho}$) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ = 25.97, 33.36, 41.92, 49.11, 52.15, 127.47, 128.87, 129.64, 145.81, 155.80 ppm. M ($C_{18}H_{21}N_5 \times HCl$) = 343.86. MS (CI, CH_5^+) m/z (%): 308 (100, $[M+H]^+$), 193 (39). HRMS (ESI^+): $[M+H]^+$ calcd for $C_{18}H_{22}N_5$, 308.1869; found: 308.1868.

4.1.3.8. (3,3-Diphenylallyl)-[1-(1,1,3,3-tetramethylbutyl)-1*H*-tetrazol-5-ylmethyl]amine (51). According to **GP1** starting from paraformaldehyde (59.3 mg, 1.98 mmol), 3,3-diphenylallylamine (807.0 mg, 3.856 mmol), 1,1,3,3-tetramethylbutyl isocyanide (265.2 mg, 1.905 mmol, 334.0 μL) and trimethylsilyl azide (219.0 mg, 1.901 mmol, 250.0 μL). Purification by CC (*n*-pentane/EtOAc = 35:65 und 1:1) yielded 91% of **51**.

Compound ***51***. Yellow oil (701.7 mg, 91%). R_f = 0.38 (*n*-pentane/EtOAc = 1:1). IR: 3321, 3022, 2954, 1953, 1678, 1598, 1492, 1444, 1234, 1114, 759, 701 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.76 (s, 9H, $(CH_3)_3$), 1.79 (s, 6H, $NC(CH_3)_3$), 1.97 (s, 2H, $CH_2C(CH_3)_3$), 2.41 (s_{br}, 1H, NH), 3.47 (d, J = 6.9 Hz, 2H, $HNCH_2CH$), 4.11 (s, 2H, CCH_2NH), 6.19 (t, J = 6.9 Hz, 1H, CH_2CH = C), 7.14–7.18 (m, 2H, $H_{ar,ortho}$), 7.20–7.40 (m, 8H, H_{ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 29.92, 30.63, 31.61, 44.18, 48.34, 53.15, 64.67, 125.91, 127.42, 127.48, 127.51, 128.19, 128.29, 129.67, 139.22, 141.77, 144.66, 153.04 ppm. M ($C_{25}H_{33}N_5$) = 403.58. MS (CI, CH_5^+) m/z (%): 404 (3, $[M+H]^+$), 191 (31), 208 (10), 193 (100). HRMS (EI, 70 eV): M^+ calcd for $C_{25}H_{33}N_5$, 403.27360; found: 403.27476.

4.1.3.9. (3,3-Diphenylallyl)-(1*H*-tetrazol-5-ylmethyl)amine hydrochloride (68·HCl). Deprotection of **51** (195.6 mg, 0.4846 mmol) according to **GP2** using HCl_{EtOH} (~1.25 M, 7.5 mmol, 6.0 mL, 15 equiv). Reaction time 8 h.

Compound ***68***. HCl. Pale yellow solid (128.8 mg, 81%); mp 142 °C dec. IR: 3433, 3057, 2944, 2735, 2647, 2468, 2345, 1773, 1627, 1446, 1110, 1053, 759, 706 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ = 3.94 (d, J = 7.1 Hz, 2H, CH_2CH=), 4.61 (s, 2H, $HNCH_2C$), 6.20 (t_{br}, J = 7.1 Hz, 1H, CH=C), 7.15–7.20 (m, 2H, $H_{ar,ortho}$), 7.25–7.30 (m, 2H, $H_{ar,ortho}$), 7.30–7.35 (m, 3H, $H_{ar,meta}$, $H_{ar,para}$), 7.38–7.47 (m, 3H, $H_{ar,meta}$, $H_{ar,para}$) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ = 41.28, 47.78, 117.59, 128.75, 129.51, 129.53, 129.70, 129.86, 130.61, 139.22, 142.03, 151.44, 155.84 ppm. M ($C_{17}H_{17}N_5 \times HCl$) = 327.82. MS (CI, CH_5^+) m/z (%): 292 (6, $[M+H]^+$), 193 (100), 113 (40), 101 (60). HRMS (ESI^+): $[M+H]^+$ calcd for $C_{17}H_{18}N_5$, 292.1556; found: 292.1556.

4.1.3.10. (4,4-Diphenylbut-3-en-1-yl)-[1-(1,1,3,3-tetramethylbutyl)-1*H*-tetrazol-5-ylmethyl]amine (54). According to **GP1** starting from paraformaldehyde (15.3 mg, 0.509 mmol), 4,4-diphenylbut-3-en-1-ylamine (225.0 mg, 1.008 mmol), 1,1,3,3-tetramethylbutyl isocyanide (69.9 mg, 0.502 mmol, 88.0 μL) and $TMSN_3$ (57.8 mg, 0.502 mmol, 66.0 μL). Purification by CC (*n*-pentane/EtOAc = 1:1) yielded **54**.

Compound ***54***. Pale yellow oil (171.9 mg, 82%). R_f = 0.32 (*n*-pentane/EtOAc = 1:1). IR: 3329, 3022, 2952, 2917, 1950, 1597, 1444, 1113, 761, 700 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 0.75 (s, 9H, $(CH_3)_3$), 1.79 (s, 7H, $NC(CH_3)_3$, NH), 1.97 (s, 2H, $CH_2C(CH_3)_3$), 2.36 (q, J = 7.1 Hz, 2H, CH_2CH), 2.82 (t, J = 7.0 Hz, 2H, CH_2CH_2NH), 4.11 (s, 2H, NCH_2C), 6.10 (t, J = 7.4 Hz, 1H, CH_2CH), 7.15–7.19 (m, 2H, $H_{ar,ortho}$), 7.19–7.32 (m, 6H, H_{ar}), 7.33–7.39 (m, 2H, $H_{ar,meta}$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ = 29.94, 30.20, 30.66, 31.61, 44.67, 49.51, 53.24, 64.69, 126.46, 127.08, 127.21, 128.11, 128.27, 129.82, 139.87, 142.34, 143.61, 153.21 ppm. M ($C_{26}H_{35}N_5$) = 417.60. MS (CI, CH_5^+) m/z (%): 418 (15, $[M+H]^+$), 306 (100), 112 (36). HRMS (EI, 70 eV): M^+ calcd for $C_{26}H_{35}N_5$, 417.28925; found: 417.28989. $C_{26}H_{35}N_5$ Anal. calcd for C, 74.78; H, 8.45; N, 16.77. Found: C, 74.60; H, 8.70; N, 16.79.

4.1.3.11. (4,4-Diphenylbut-3-en-1-yl)-(1*H*-tetrazol-5-ylmethyl)amine hydrochloride (69·HCl). According to **GP2** starting from **54** (91.7 mg, 0.220 mmol) and HCl_{EtOH} (~1.25 M, 3.0 mL, 3.8 mmol, 17 equiv). Reaction time: 9 h.

Compound ***69***. HCl. Colorless solid (58.3 mg, 78%); mp 158 °C. IR: 3055, 2927, 2754, 2684, 1556, 1457, 2362, 1956, 1889, 1598, 1555, 1443, 1053, 866, 765, 703 cm^{-1}. ^1H NMR (500 MHz, CD_3CD_2OD) δ = 2.59–2.67 (m, 2H, CH_2CH), 3.30–3.37 (m, 2H, NCH_2CH_2), 4.62 (s, 2H, CCH_2NH), 6.12 (t, J = 7.3 Hz, 1H, CH=C), 7.15–7.19 (m, 2H, $H_{ar,ortho}$), 7.19–7.27 (m, 5H, H_{ar}), 7.31–7.36 (m, 1H, $H_{ar,para}$), 7.38–7.43 (m, 2H, $H_{ar,meta}$) ppm. ^{13}C NMR (125 MHz, CD_3CD_2OD) δ = 27.47, 41.62, 48.34, 122.86, 128.08, 128.31, 128.36, 128.92, 129.40, 130.41, 140.28, 142.68, 146.71, 155.28 ppm. M ($C_{18}H_{19}N_5 \times HCl$) = 341.85. MS ($ESI^+$) m/z: 611 ($[2\ M+H]^+$), 306 ($[M+H]^+$). HRMS (ESI^+): $[M+H]^+$ calcd for $C_{18}H_{20}N_5$, 306.1713; found: 306.1711.

4.1.3.12. (3-Carbazol-9-ylpropyl)-1-[1-(1,1,3,3-tetramethylbutyl)-1*H*-tetrazol-5-ylmethyl]amine (57). According to **GP1** starting from paraformaldehyde (30.4 mg, 1.01 mmol), 3-carbazol-9-

ylpropylamine (225.8 mg, 1.007 mmol), 1,1,3,3-tetramethylbutyl isonitrile (139.7 mg, 1.004 mmol, 176.0 μL) and $TMSN_3$ (115.6 mg, 1.004 mmol, 132.0 μL). Purification by CC (*n*-pentane/EtOAc = 6:4).

Compound ***57***. Colorless solid (236.3 mg, 56%); mp 153 °C. R_f = 0.16 (*n*-pentane/EtOAc = 6:4). IR: 3316, 3053, 2957, 1897, 1626, 1593, 1484, 1454, 1326, 837, 754, 727 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 0.66 (s, 9H, $(CH_3)_3$), 1.69 (s, 6H, $NC(CH_3)_2$), 1.70 (s_{br}, 1H, NH) 1.83 (s, 2H, $CH_2C(CH_3)_3$), 2.02 (p, *J* = 6.7 Hz, 2H, $CH_2CH_2CH_2$), 2.62 (t, *J* = 6.7 Hz, 2H, CH_2CH_2NH), 4.00 (s, 2H, CCH_2NH), 4.38 (t, *J* = 6.7 Hz, 2H, CH_2NC), 7.15 (ddd, *J* = 7.8/5.1/2.8 Hz, 2H, NCCHCHCH), 7.34–7.41 (m, 4H, NCCH, NCCHCH), 8.02 (dt, *J* = 7.8/0.9 Hz, 2H, NCCCH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ = 29.54, 30.21, 30.84, 31.81, 40.68, 45.38, 47.03, 53.41, 64.67, 108.84, 119.08, 120.57, 123.05, 125.87, 140.63, 153.61 ppm. M ($C_{25}H_{34}N_6$) = 418.59. MS (CI, CH_5^+) *m*/*z* (%): 419 (52, $[M+H]^+$), 307 (100). HRMS (EI, 70 eV): M^+ calcd for $C_{25}H_{34}N_6$, 418.2845; found: 418.2849. Anal. calcd for C, 71.74; H, 8.19; N, 20.08. Found: C, 71.38; H, 8.23; N, 19.86.

4.1.3.13. (3-Carbazol-9-ylpropyl)-(1*H*-tetrazol-5-ylmethyl)amine hydrochloride (70·HCl). Deprotection of **57** (156.6 mg, 0.3693 mmol) according to **GP2** in HCl_{EtOH} (~1.25 M, 6.3 mmol, 5.0 mL, 17 equiv). Reaction time 3 h reflux, 16 h rt. In deviation from **GP2**, the product was purified by prep. MPLC (RP18, CH_3CN/H_2O = 1:9–10:0, UV 220 nm). After chromatographic purification the product was dissolved in an excess of HCl_{EtOH}. The solvent was removed in vacuo and **68**·HCl was obtained as a pale yellow solid.

Compound ***70***. HCl. Pale yellow solid (120.7 mg, 95%); mp 98–101 °C (dec.). IR: 3184, 2948, 2811, 1450, 1911, 1771, 1625, 1595, 1548, 1483, 1453, 1325, 1231, 1222, 1042, 743, 722 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ = 2.29–2.39 (m, 2H, $CH_2CH_2CH_2$), 3.17–3.27 (m, 2H, CH_2CH_2NH), 4.50–4.59 (m, 4H, NCH_2C, CH_2CH_2N), 7.22 (t, *J* = 7.3 Hz, 2H, NCCHCHCH), 7.47 (t, *J* = 7.6 Hz, 2H, NCCHCH), 7.56 (d, *J* = 7.6 Hz, 2H, NCCHCHCH), 8.09 (d, *J* = 7.5 Hz, 2H, NCCH) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ = 26.88, 40.61, 41.92, 46.98, 109.81, 120.38, 121.32, 124.31, 127.05, 141.55, 155.72 ppm. M ($C_{17}H_{18}N_6 \times HCl$) = 342.83. MS (EI, 70 eV) *m*/*z* (%): 306 (38, $[M]^+$), 180 (89), 66 (100). HRMS (ESI^+): $[M+H]^+$ calcd for $C_{17}H_{19}N_6$, 307.1666; found: 307.1664.

4.1.3.14. 4-[1-(1,1,3,3-Tetramethylbutyl)-1*H*-tetrazol-5-ylmethyl]piperazine-1-carboxylic acid-tert-butylester (59). According to **GP1** starting from paraformaldehyde (57.2 mg, 1.91 mmol), 1-Boc-piperazine (352.1 mg, 1.890 mmol), 1,1,3,3-tetramethylbutyl isocyanide (266.0 mg, 1.910 mmol, 335.0 μL) and $TMSN_3$ (219.9 mg, 1.908 mmol, 251.0 μL). Purification by CC (*n*-pentane/EtOAc = 1:1) gave **59**.

Compound ***59***. Colorless solid (586.3 mg, 82%); mp 129 °C. R_f = 0.36 (*n*-pentane/EtOAc = 1:1). IR: 2982, 2962, 1683, 1432, 1365, 1277, 1182, 1128, 999, 766 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 0.83 (s, 9H, $C(CH_3)_3$), 1.46 (s, 9H, $OC(CH_3)_3$), 1.85 (s, 6H, $NC(CH_3)_2$), 2.10 (s, 2H, $CH_2C(CH_3)_3$), 2.50 (s_{br}, 4H, NCH_2CH_2NCO), 3.42 (s_{br}, 4H, CH_2NCO), 3.88 (s, 2H, CCH_2N) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ = 28.57, 29.91, 30.98, 31.86, 43.35, 52.49, 53.08, 53.68, 65.82, 80.14, 150.90, 154.85 ppm. M ($C_{19}H_{36}N_6O_2$) = 380.54. MS (CI, CH_5^+) *m*/*z* (%): 381 (81, $[M+H]^+$), 325 (38), 269 (84), 270 (12), 213 (100). HRMS (EI, 70 eV): M^+ calcd for $C_{19}H_{36}N_6O_2$, 380.2900; found: 380.2901. Anal. calcd for C, 59.97; H, 9.54; N, 22.08. Found: C, 59.86; H, 9.44; N, 22.14.

4.1.3.15. 1-[1-(1,1,3,3-Tetramethylbutyl)-1*H*-tetrazol-5-ylmethyl]piperazine (62). Compound **59** (63.1 mg, 0.166 mmol) was treated with HCl (~0.5 M in EtOH, 1.0 mL, 0.5 mmol) and stirred at rt. After a reaction time of 7 h the solvent was removed in vacuo. The resulting crude product was taken up in aqueous NaOH (2 M, 3 mL) and extracted with Et_2O (3 × 4 mL). The combined organic phases were dried (Na_2SO_4) and evaporated to dryness, to yield a colorless solid.

Compound ***62***. Colorless solid (46.0 mg, 99%); mp 89–91 °C. IR: 3302, 2947, 2806, 1464, 1394, 1229, 1120, 823, 806 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 0.82 (s, 9H, $(CH_3)_3$), 1.66 (s_{br}, 1H, NH), 1.84 (s, 6H, $(NC(CH_3)_2)$, 2.09 (s, 2H, $CH_2C(CH_3)_3$), 2.48 (s_{br}, 4H, NCH_2CH_2NH), 2.86 (t, *J* = 4.8 Hz, 4H, NCH_2CH_2NH), 3.82 (s, 2H, CCH_2N) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ = 29.62, 30.81, 31.66, 45.77, 52.86, 53.35, 54.41, 65.66, 150.92 ppm. M ($C_{14}H_{28}N_6$) = 280.42. MS (CI, CH_5^+) *m*/*z* (%): 281 (32, $[M+H]^+$), 169 (100). HRMS (EI, 70 eV): M^+ calcd for $C_{14}H_{28}N_6$, 280.2376; found: 280.2400.

4.1.3.16. 1-(1*H*-Tetrazol-5-ylmethyl)piperazine dihydrochloride (73·2HCl). Compound **59** (185.4 mg, 0.4872 mg) was treated with HCl_{EtOH} (~1.25 M, 18.8 mmol, 15.0 mL, 19 equiv) and stirred for 29 h at 40 °C. In doing so, **73**·2HCl precipitated and could be isolated by filtration.

Compound ***73***. 2HCl. Colorless solid (107.3 mg, 91%); mp 236 °C dec IR: 3423, 3017, 3000, 2949, 2726, 2366, 2345, 1563, 1458, 1430, 1388, 1210, 1165, 1063, 1026, 968, 858 cm^{-1}. ^1H NMR (500 MHz, D_2O) δ = 3.41–3.48 (m, 4H, CH_2CH_2), 3.48–3.56 (m, 4H, CH_2CH_2), 4.63 (s, 2H, CCH_2N) ppm. ^{13}C NMR (125 MHz, D_2O) δ = 42.13, 49.30, 49.64, 152.49 ppm. M ($C_6H_{12}N_6 \times 2HCl$) = 241.12. MS (CI, CH_5^+) *m*/*z* (%): 169 (100, $[M+H]^+$), 145 (17), 127 (18). HRMS (ESI^+): $[M+H]^+$ calcd for $C_6H_{13}N_6$, 169.1196; found: 169.1197.

The TFA-salt 73·2TFA is described in literature.[49]

4.1.3.17. 1*H*-Tetrazol-5-ylmethylamine hydrochloride (19·HCl)[50,51]. Triethylsilane (0.73 g, 6.3 mmol, 1.0 mL, 11 equiv) was slowly added to a solution of **39** (220.8 mg, 0.5848 mmol) in TFA (4.0 mL). The reaction mixture was refluxed for 4.5 h. After cooling to rt, H_2O (10 mL) was added and the aqueous phase was washed with Et_2O (3 × 10 mL). The crude product was purified by ion exchange chromatography (Amberlite IRA 410, strongly basic and (Amberlite IR 120, strongly acidic ion exchange resin).

Compound ***19***. HCl. 43.4 mg (70%). Colorless solid; mp: 138 °C (Lit: 143 °C[50], 138–141 °C[51]). IR: 3422, 3161, 1718, 1636, 1404, 1091, 945, 801 cm^{-1}. ^1H NMR (400 MHz, D_2O) δ = 4.63 (s, 2H, CH_2) ppm. ^{13}C NMR (100 MHz, D_2O) δ = 33.61, 153.40 ppm. M ($C_2H_5N_5 \times HCl$) = 135.56. MS (FAB^+) *m*/*z* (%): 100 ($[M+H]^+$). HRMS (FAB^+): $[M+H]^+$ ber. für $C_2H_6N_5$, 100.0623; gef. 100.0618.

4.2. GABA uptake assays

[^{3}H]GABA uptake was determined as reported.[52]

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Supplementary data

Supplementary data (Experimental data for compounds **25**, **39**, **40**, **42**, **44**, **46**, **47**, **49**, **52**, **53**, **55**, **56**, **58**, **60**, **61**, **63**. **2**HCl, **64**·HCl, **65**·HCl, **71**·HCl, **72**·HCl and **74**. **2**HCl) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.08.039.

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