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L-type amino acid transporter 1 activity of 1,2,3-triazolyl analogs of L-histidine and L-tryptophan

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Abstract

A series of 1,2,3-triazole analogs of the amino acids L-histidine and L-tryptophan were modeled, synthesized and tested for L-type amino acid transporter 1 (LAT1; SLC7A5) activity to guide the design of amino acid-drug conjugates (prodrugs). These triazoles were conveniently prepared by the highly convergent Huisgen 1,3-dipolar cycloaddition (Click Chemistry). Despite comparable predicted binding modes, triazoles generally demonstrated reduced cell uptake and LAT1 binding potency relative to their natural amino acid counterparts. The structure-activity relationship (SAR) data for these triazoles has important ramifications for treating cancer and brain disorders using amino acid prodrugs or LAT1 inhibitors.

Graphical Abstract

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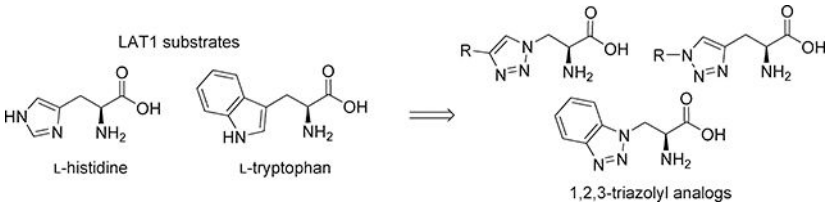
Conflicts of interest

The authors confirm no conflict of interest.

Appendix A. Supplementary data

Full experimental details, compound characterization data, including ^{1}H and ^{13}C NMR spectra for newly synthesized compounds **7d** and **11d**, and ligand docking description along with models of compounds **7e**, **11e** and **17** docked in the LAT1 active site can be found in the online version.



Keywords

membrane transporter; solute carrier family; triazole; click chemistry; amino acid; cancer; blood-brain barrier

Conjugation of drugs with amino acids (“amino acid prodrugs”) has become an increasingly common strategy for targeted drug delivery across the blood-brain barrier (BBB) and into cancer cells by way of the L-type amino acid transporter 1 (LAT1; SLC7A5). LAT1 is a sodium-independent membrane solute carrier protein[1–3] found in several tissues throughout the body, with high levels of expression in brain,[4, 5] spleen, testis, placenta, thymus, skeletal muscle and various cancer cells.[6–10] LAT1 transports lipophilic amino acids such as phenylalanine, leucine, isoleucine, methionine, tryptophan as well as thyroid hormones including thyroxine. Additionally, LAT1 transports the hydrophilic amino acid histidine. We and others have shown that LAT1 can also transport aryl-substituted phenylalanine analogs containing various functional groups,[2, 11–13] as well as amino acid bioisosteres such as hydroxamic acids and esters.[14] Given these findings that LAT1 is relatively tolerant of structural changes to the amino acid promoiety, we sought an amino acid scaffold that would be amenable to the rapid assembly of prodrugs while maintaining substrate activity.

The Huisgen 1,3-dipolar cycloaddition, also known as “Click Chemistry”,[15–20] provides a synthetic strategy for connecting an amino acid moiety to a drug molecule via a 1,2,3-triazole ring. Specifically, we envisioned using Click Chemistry to attach an alkyne- or azide-substituted amino acid to a drug molecule possessing a biodegradable functional group that is linked to an azide or alkyne moiety (Figure 1). These 1,2,3-triazoles (hereafter referred to as “triazoles”) are structurally similar to the amino acids L-histidine and L-tryptophan. Thus, we hypothesized that triazoles would be LAT1 substrates based on the similarity of their predicted binding modes to these endogenous ligands (supplementary data). Moreover, L-histidine is one of the most potent LAT1 substrates,[12, 21] so we thought triazole analogs would also have good potency. Click Chemistry offers the additional advantage of obtaining a single triazole regioisomer. Prior to using this strategy for designing amino acid prodrugs, we wanted to test its feasibility by determining the structure-activity relationship (SAR) for a series of triazole structural analogs that we have designated as *N*-linked or *C*-linked based on which triazole atom is connected to the amino acid beta carbon (Figure 1). We varied both the size and polarity of the triazole substituent R to compare with the SAR of structurally-related histidine analogs and tryptophan. Compounds were evaluated in *trans*-stimulation and *cis*-inhibition cell assays (as described below).

Scheme 1 shows the synthesis of azide intermediate **4** used to prepare *N*-linked triazoles. Starting with commercially available Boc-protected serine **1**, we prepared *tert*-butyl ester **2**

using *tert*-butyl (*Z*)-*N*,*N*′-diisopropylcarbamimidate.[22] We were unable to prepare a tosylate of alcohol **2** by reaction with tosyl chloride presumably due to steric crowding of the Boc and *tert*-butyl ester groups. Instead we prepared benzyl sulfonate **3** as previously described.[23] Subsequent nucleophilic substitution with sodium azide using tetrabutylammonium bromide as a phasetransfer agent provided the desired azide **4**[23] in moderate yield (60%).

Using methodology similar to what was previously published for preparing triazoles **7ac**,[24] cycloaddition of azide **4** with various substituted alkynes (**5a-e**) was catalyzed by copper(I) ion generated *in situ* from sodium ascorbate reduction of copper(II) acetate to give protected triazole analogs **6a-e** in 35–100% yields (Scheme 2). Both the *tert*-butyl ester and Boc protecting groups were conveniently removed in one step using trifluoroacetic acid (TFA) in the presence of carbocation scavenger triethylsilane (Et_3SiH). Additionally, Et_3SiH has been shown to increase the rate of Boc deprotection.[25] These deprotection conditions were also used in the synthesis of *C*-linked triazole **11d** (Scheme 3) and benzotriazole **13** (Scheme 4).

Scheme 3 illustrates the synthesis of the two *C*-linked triazoles **11d** and **11e**. Benzyl triazole **11e** was prepared from benzyl azide **8e** and unprotected, commercially available (*S*)-2-aminopent-4-ynoic acid using Click Chemistry conditions “A” as had been applied in the synthesis of *N*-linked triazoles of Scheme 2. As **11e** was chronologically the first triazole analog we synthesized in this project, we had hoped to avoid using protecting groups. However, we found that separation of the amino acid **11e** from the other reactants (i.e. diacetoxycopper and sodium ascorbate) proved challenging; thus, we modified our synthesis to use protected amino acids in the Click Chemistry reactions for all other analogs of Schemes 2–4. Hence, alkyne **9**[26] was prepared by Boc and *tert*-butyl ester protection of (*S*)-2-aminopent-4-ynoic acid. For unknown reasons, we found that methyl azide **8d** (prepared *in situ* from sodium azide and iodomethane) did not react with alkyne **9** using the same conditions as for *N*-linked triazoles of Scheme 2. Alternative conditions[27] “B” employing CuI and triethylamine (Et_3N) were found to give desired **10d** in 60% yield. Methyl triazole **11d** was obtained following deprotection of **10d** using TFA and Et_3SiH.

Benzotriazole **12** was prepared by a [3+2] cycloaddition of azide **4** and benzyne generated *in situ* by a cesium fluoride mediated elimination of 2-(trimethylsilyl)phenyl triflate (Scheme 4; 72% yield).[28] Desired amino acid **13** was obtained by deprotection of **12** with TFA and Et_3SiH, as performed for triazoles of Scheme 2.

Triazoles of Schemes 2–4 were tested for LAT1 activity as previously described (24-well poly-D plate configuration)[12, 29] using human embryonic kidney cells containing inducible LAT1 (HEK-hLAT1)[30] in two different assays: *cis*-inhibition and *trans*-stimulation, to determine ligand potency and relative transport rates, respectively. In the *cis*-inhibition assay, test compounds were incubated with cells in the presence of a known substrate, [^{3}H]-gabapentin (6 nM). Compounds were screened at 200 μM to determine %inhibition of [^{3}H]-gabapentin uptake relative to a LAT1 inhibitor BCH (2 mM),[31] set to 100% inhibition. Select compounds were then tested at various concentrations to determine the half maximal inhibitory concentration (IC_{50}). *cis*-Inhibition indicates ligand potency, however, it does not distinguish between LAT1 inhibitors and substrates. To identify whether a compound was a substrate, we performed a *trans*stimulation assay. This experiment is possible due to the

alternating access mechanism of LAT1,[32] in which an exchange occurs between intra- and extra-cellular amino acids in a 1:1 mole ratio. The assay is performed by loading cells with [^{3}H]-gabapentin prior to exposure to test compounds. The efflux rate of [^{3}H]-gabapentin (fmol/min) was calculated at 3 minutes after adding test compound. % Efflux was normalized relative to L-phenylalanine **18**, which had an efflux rate of 2.7 ± 0.3 fmol/min, from an average of seven experiments.

Positive controls included natural amino acid substrates L-histidine **15**, L-phenylalanine **18** and L-tryptophan **22**. Non-substrate L-arginine **14** was used as a negative control, which had a relative efflux rate of 28% in our *trans*-stimulation assay. Non-substrates generally show 20−35% relative efflux due to background levels of exported [^{3}H]-gabapentin. Low levels of endogenous amino acids present in the cell assay may cause efflux via LAT1 or an alternate transporter.

Unfortunately, triazole analogs of Table 1 (**7a-e**, **11d-e**) were considerably less potent (lower %inhibition and greater IC_{50} values) in our *cis*-inhibition assay than related histidine analogs (**15–17**) of Table 2. Unsubstituted *N*-linked triazole **7a** (R = H) showed the highest relative efflux rate and greatest potency of the triazole series. However, both the potency (160 μM vs. 110 μM) and relative %efflux (82% vs. 140%) for **7a** were inferior to L-histidine **15**. In contrast to histidine analogs **16–17**, substitution in the triazole series was less well tolerated. Only a methyl substituent in compound **7d** displayed comparable %efflux to its related histidine analog **16**, but showed a 2-fold decrease in potency (440 μM vs. 190 μM). Moreover, benzyl substitution in triazoles **7e** and **11e** resulted in loss of potency relative to analog **17**. Though one could interpret the negative %inhibition for **7e** and **11e** as due to LAT1 activation for [^{3}H]gabapentin uptake, we think it is possible that a change in plasma membrane integrity could cause an uptake signal increase compared with control in the absence of any test compounds. Nonetheless, these negative %inhibition values do not change the interpretation of our data, that triazoles **7e** and **11e** have poor potency. However, triazoles of Table 1 (**7a-e**, **11d-e**) demonstrated relative %efflux greater than our negative control L-arginine **14** (37–82% vs. 28%), suggesting that they are being transported into cells by LAT1.

It should be noted that we were unable to prepare the *C*-linked analog of triazole **7a** by reaction of TMS-azide with alkyne **9** using either conditions A or B of Scheme 3. An alternate, multi-step synthesis (not involving Click Chemistry) of this unsubstituted *C*-linked triazole in racemic form has previously been described;[33] however, given the lack of potency for *C*-linked triazoles **11d** and **11e**, we decided not to pursue this compound.

Relative to triazoles of Table 1, benzotriazole **13** (Table 2) demonstrated improved %efflux and potency. Additionally, it appeared to be a better substrate in terms of %efflux compared with the natural substrate tryptophan **22** (100% vs. 59%), albeit with a 2-fold decrease in potency. Thus, all of the triazole analogs of Tables 1 and 2 had diminished potency relative to their corresponding histidine and tryptophan analogs.

Based on our models, both triazoles and the corresponding histidine analogs dock within a model of the LAT1 binding site in a comparable manner (supplementary data). Therefore,

we cannot explain the difference in activity based on their predicted binding modes. Furthermore, comparing cLogP values for triazoles with their histidine congeners did not provide any additional insight into why triazoles have decreased potency. For example, *N*-linked triazoles (**7a-7e**) exhibit an almost order of magnitude higher cLogP[34] than their histidine counterparts (e.g. cLogP: **7a** = −2.9 vs. **15** = −3.7). Whereas, *C*-linked triazoles, which were even less potent, had more negative cLogP values (e.g. **11d** = −4.2 vs. **16** = −3.7). Moreover, pyridyl analogs **19–21**, which we previously reported as potent LAT1 substrates,[12] had a comparable cLogP to triazole **7a** (−3.1 vs. −2.9, respectively). The only apparent difference between these triazoles and their related histidine and pyridyl analogs is heterocycle basicity. A 1,2,3-triazole ring is non-basic with a reported pK_a of 1.15 for its conjugate acid.[35] However, our models, representing snapshots with the highly dynamic transport cycle do not indicate the presence of acidic side chains in the vicinity of the ligand binding site. Thus, we cannot determine the potential functional role of a basic heterocycle (i.e. imidazole and pyridine). Furthermore, imidazole and pyridine rings would be primarily neutral under the conditions of our cell assay (pH 7.4), even though their protonation states inside the binding site is unknown. Given the fact that most natural LAT1 substrates do not contain a basic heterocycle (e.g. phenylalanine **18,** tryptophan **22**, leucine, methionine, tyrosine, etc.), it is unclear to us why triazoles have decreased potency compared with structurally-related histidine and pyridyl analogs.

A series of substituted 1,2,3-triazolyl amino acids were synthesized and tested for LAT1 substrate activity. The synthesis of these triazole analogs is convenient, potentially serving as a template for conjugation of drug molecules with amino acids (i.e. hypothetical prodrugs of Figure 1) via the highly convergent Huisgen 1,3-dipolar cycloaddition. However, only a benzotriazole analog **13** exhibited a better uptake rate than the corresponding natural amino acid analog L-tryptophan **22**, and all of the triazoles of the current work were less potent than their corresponding histidine and tryptophan equivalents. Despite having poor potency for inhibiting LAT1 uptake of [^{3}H]-gabapentin, triazoles may be weak binding substrates, as evidenced by their activity in a *trans*-stimulation assay relative to non-substrate L-arginine **14**. Though we have no evidence to explain why a basic heterocycle (e.g. imidazole and pyridine) provides greater potency than these non-basic triazoles, our results suggest that the binding site is sensitive to relatively subtle changes in ligand properties. Moreover, it was previously noted that LAT1 is selective for aromatic amino acids relative to aliphatic ones, and this preference does not correlate well with lipophilicity.[36, 37] Our data expand upon that observation by showing that aromaticity does not guarantee good ligand binding. Thus, for reasons that are not obvious to us, amino acids containing a 1,2,3-triazole ring appear to be inferior LAT1 substrates compared with histidine and tryptophan structural analogs. These findings could influence the design of amino acid containing prodrugs as well as LAT1 inhibitors for treating brain disorders and cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References and notes

1. Kanai Y; Segawa H; Miyamoto K; Uchino H; Takeda E; Endou H J. Biol. Chem 1998, 273, 23629. [PubMed: 9726963]
2. Uchino H; Kanai Y; Kim DK; Wempe MF; Chairoungdua A; Morimoto E; Anders MW; Endou H Mol. Pharmacol 2002, 61, 729. [PubMed: 11901210]
3. del Amo EM; Urtti A; Yliperttula M Eur. J. Pharm. Sci 2008, 35, 161. [PubMed: 18656534]
4. Boado RJ; Li JY; Nagaya M; Zhang C; Pardridge WM Proc. Natl. Acad. Sci. USA 1999, 96, 12079. [PubMed: 10518579]
5. Kido Y; Tamai I; Uchino H; Suzuki F; Sai Y; Tsuji A J. Pharm. Pharmacol 2001, 53, 497. [PubMed: 11341366]
6. Yanagisawa N; Satoh T; Hana K; Ichinoe M; Nakada N; Endou H; Okayasu I; Murakumo Y Cancer Biomark. 2015, 15, 365. [PubMed: 25835180]
7. Kobayashi H; Ishii Y; Takayama T J. Surg. Oncol 2005, 90, 233. [PubMed: 15906366]
8. Ebara T; Kaira K; Saito J; Shioya M; Asao T; Takahashi T; Sakurai H; Kanai Y; Kuwano H; Nakano T Anticancer Res. 2010, 30, 4223. [PubMed: 21036745]
9. Ichinoe M; Mikami T; Yoshida T; Igawa I; Tsuruta T; Nakada N; Anzai N; Suzuki Y; Endou H; Okayasu I Pathol. Int 2011, 61, 281. [PubMed: 21501294]
10. Takeuchi K; Ogata S; Nakanishi K; Ozeki Y; Hiroi S; Tominaga S; Aida S; Matsuo H; Sakata T; Kawai T Lung Cancer 2010, 68, 58. [PubMed: 19559497]
11. Ylikangas H; Malmioja K; Peura L; Gynther M; Nwachukwu EO; Leppanen J; Laine K; Rautio J; Lahtela-Kakkonen M; Huttunen KM; Poso A ChemMedChem 2014, 9, 2699. [PubMed: 25205473]
12. Chien HC; Colas C; Finke K; Springer S; Stoner L; Zur AA; Venteicher B; Campbell J; Hall C; Flint A; Augustyn E; Hernandez C; Heeren N; Hansen L; Anthony A; Bauer J; Fotiadis D; Schlessinger A; Giacomini KM; Thomas AA J. Med. Chem 2018, 61, 7358. [PubMed: 30048132]
13. Augustyn E; Finke K; Zur AA; Hansen L; Heeren N; Chien H-C; Lin L; Giacomini KM; Colas C; Schlessinger A; Thomas AA Bioorg. Med. Chem. Lett 2016, 26, 2616. [PubMed: 27106710]
14. Zur AA; Chien H-C; Augustyn E; Flint A; Heeren N; Finke K; Hernandez C; Hansen L; Miller S; Lin L; Giacomini KM; Colas C; Schlessinger A; Thomas AA Bioorg. Med. Chem. Lett 2016, 26, 5000. [PubMed: 27624080]
15. Zhao Z; Yao Z; Xu X Curr. Org. Chem 2017, 21, 2240.
16. Singh MS; Chowdhury S; Koley S Tetrahedron 2016, 72, 5257.
17. Wang C; Ikhlef D; Kahlal S; Saillard J-Y; Astruc D Coord. Chem. Rev 2016, 316, 1.
18. Schoffelen S; Meldal M Alkyne-azide reactions, 1st *ed*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2014.
19. Heravi MM; Tamimi M; Yahyavi H; Hosseinnejad T Curr. Org. Chem 2016, 20, 1591.
20. Kolb HC; Finn MG; Sharpless KB Angew. Chem. Int. Ed. Engl 2001, 40, 2004. [PubMed: 11433435]
21. Yanagida O; Kanai Y; Chairoungdua A; Kim DK; Segawa H; Nii T; Cha SH; Matsuo H; Fukushima J; Fukasawa Y; Tani Y; Taketani Y; Uchino H; Kim JY; Inatomi J; Okayasu I; Miyamoto K; Takeda E; Goya T; Endou H Biochim. Biophys. Acta 2001, 1514, 291. [PubMed: 11557028]
22. West KR; Bake KD; Otto S Org. Lett 2005, 7, 2615. [PubMed: 15957904]

23. Zou Y; Fahmi NE; Vialas C; Miller GM; Hecht SM J. Am. Chem. Soc 2002, 124, 9476. [PubMed: 12167044]
24. Gajewski M; Seaver B; Esslinger CS Bioorg. Med. Chem. Lett 2007, 17, 4163. [PubMed: 17561393]
25. Mehta A; Jaouhari R; Benson TJ; Douglas KT Tetrahedron Lett. 1992, 33, 5441.
26. McConathy J; Zhou D; Shockley SE; Jones LA; Griffin EA; Lee H; Adams SJ; Mach RH Mol. Imaging 2010, 9, 329. [PubMed: 21084029]
27. Kaur J; Bhardwaj A; Sharma SK; Wuest F Bioorg. Med. Chem 2013, 21, 4288. [PubMed: 23706267]
28. Shi F; Waldo JP; Chen Y; Larock RC Org. Lett 2008, 10, 2409. [PubMed: 18476707]
29. Geier EG; Schlessinger A; Fan H; Gable JE; Irwin JJ; Sali A; Giacomini KM Proc. Natl. Acad. Sci. USA 2013, 110, 5480. [PubMed: 23509259]
30. Zerangue NUS Patent 7,462,459 B2, 2008.
31. Segawa H; Fukasawa Y; Miyamoto K; Takeda E; Endou H; Kanai Y J. Biol. Chem 1999, 274, 19745. [PubMed: 10391916]
32. Fotiadis D; Kanai Y; Palacin M Mol. Aspects Med 2013, 34, 139. [PubMed: 23506863]
33. Ikeda Y; Kawahara S.-i.; Taki M; Kuno A; Hasegawa T; Taira K Protein Eng. 2003, 16, 699. [PubMed: 14560056]
34. cLogP was calculated using ChemDraw v.17.1.
35. Catalan J; Abboud JLM; Elguero J Adv. Heterocycl. Chem 1987, 41, 187.
36. Ylikangas H; Peura L; Malmioja K; Leppanen J; Laine K; Poso A; Lahtela-Kakkonen M; Rautio J Eur. J. Pharm. Sci 2013, 48, 523. [PubMed: 23228412]
37. Puris E; Gynther M; Huttunen J; Petsalo A; Huttunen KM J. Controlled Release 2017, 261, 93.

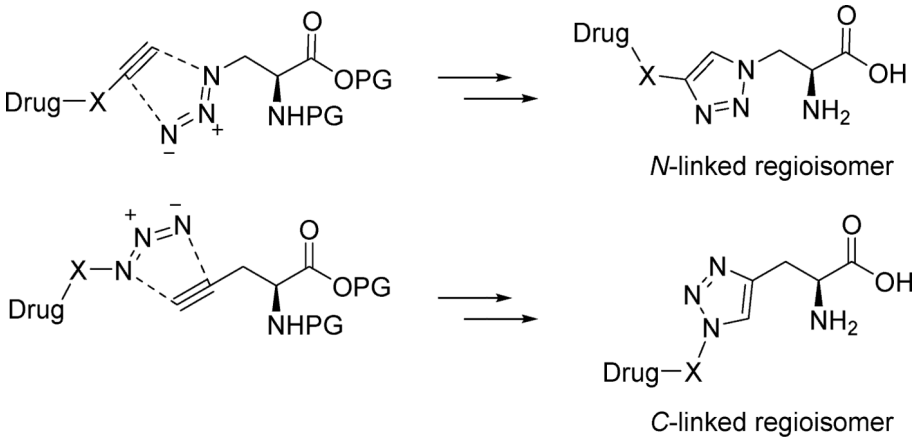
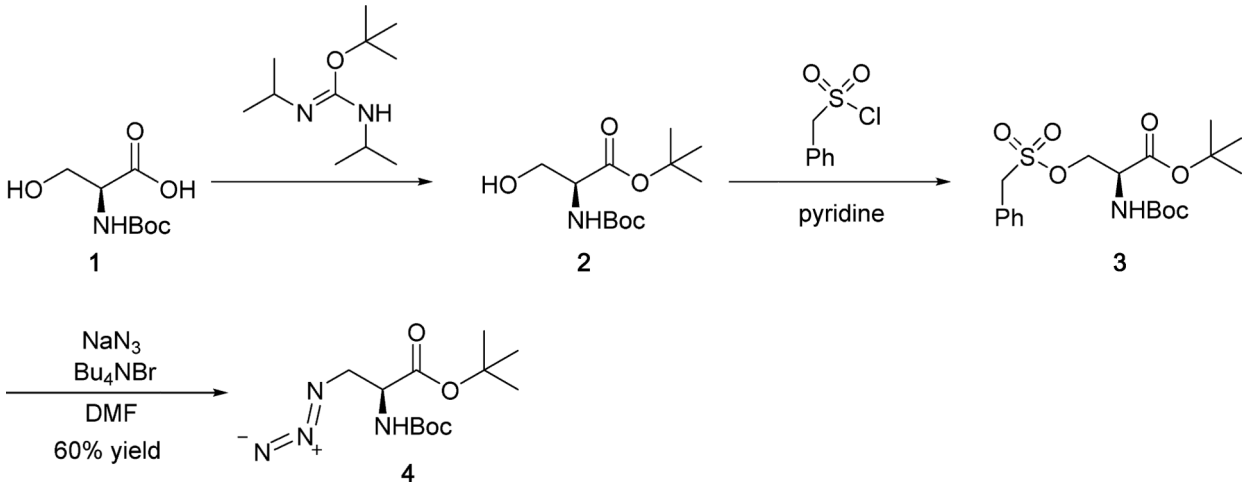
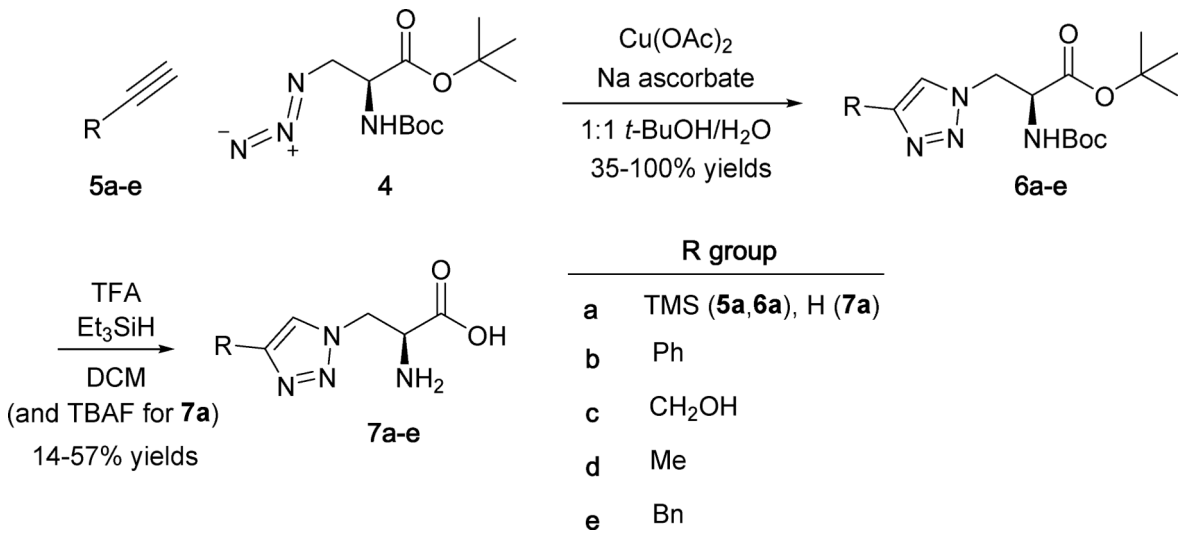


Figure 1.
Click Chemistry design strategy for *N*- and *C*-linked β−1,2,3-triazolyl amino acid prodrugs.

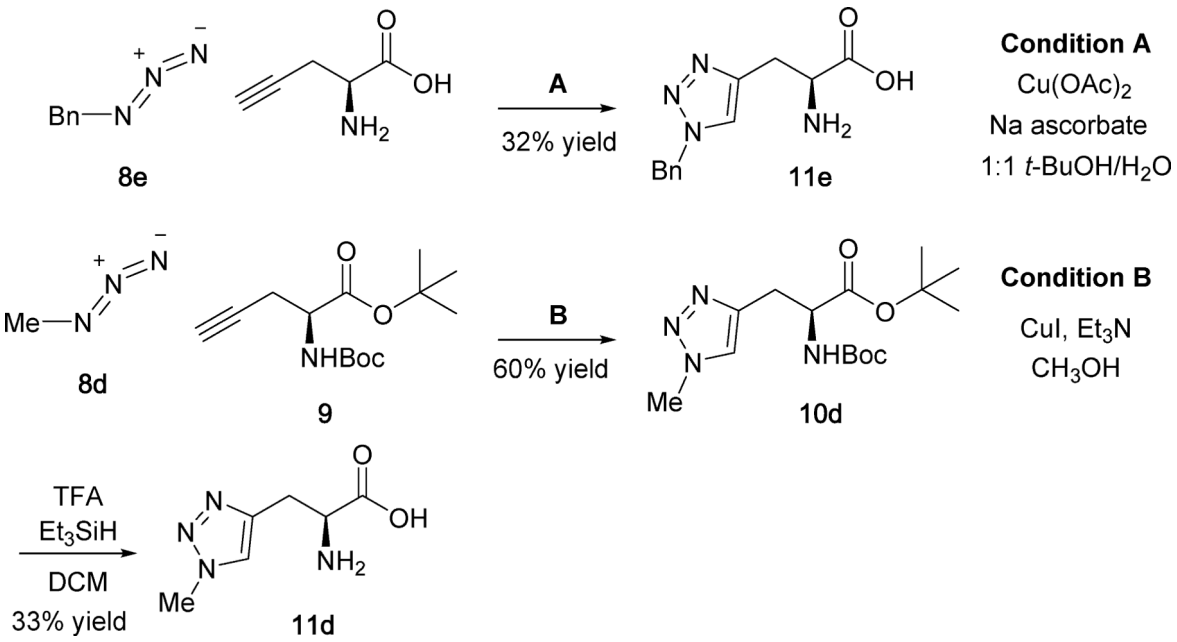


Scheme 1.
Synthesis of azide intermediate **4**.

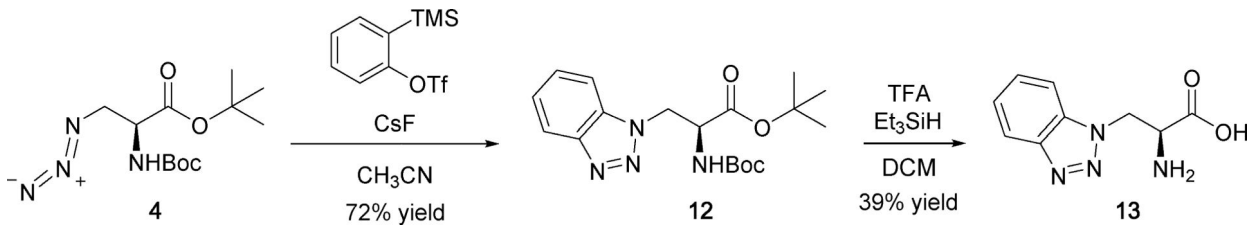


Scheme 2.

Synthesis of *N*-linked triazoles **7a-e**.



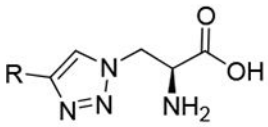
Scheme 3.
Synthesis of *C*-linked triazoles **11d-e**.



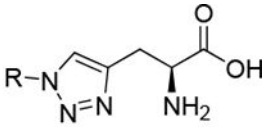
Scheme 4.
Synthesis of benzotriazole **13**.

Table1

LAT1 *trans*-stimulation and *cis*-inhibition cell assay activity for *N*- and *C*-linked triazoles **7a-e** and **11d-e**.



N-linked



C-linked

Compound[a]	R	Regioisomer	Relative %Efflux[b]	% Inhibition[c]	IC_{50} (μM)[d]
7a	H	N	82	58	160 ± 27
7b	Ph	N	37	13	>1000
7c	CH_2OH	N	57	14	>1000
7d	Me	N	70	38	440 ± 190
11d	Me	C	63	20	>1000
7e	Bn	N	49	−6	ND[e]
11e	Bn	C	43	−19	ND[e]

[a] Cell assay data was obtained at least in triplicate (24-well poly-D plate configuration) in each condition. All compounds above are single enantiomers of L configuration.

[b] Compounds were tested at 200 μM for their ability to cause efflux (fmol/min) of [^{3}H]-gabapentin from pre-loaded HEK-hLAT1 cells. Efflux of [^{3}H]-gabapentin was calculated at 3 min after adding test compound. %Efflux was normalized relative to L-Phe (**18**), which had an efflux rate of 2.7 ± 0.3 fmol/min, from an average of seven experiments.

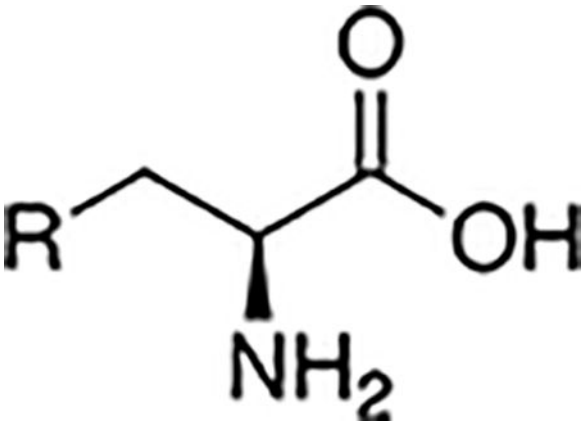
[c] Compounds were tested at 200 μM for their ability to inhibit uptake of [^{3}H]-gabapentin into HEK-hLAT1 cells. Data are presented as % inhibition relative to background signal in the absence of a test compound.

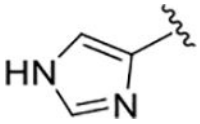
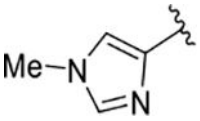
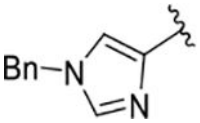
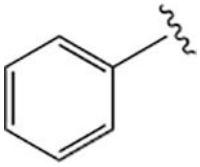
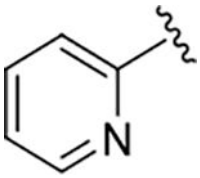
[d] For IC_{50} determinations, varying concentrations of each compound were added, from 0.1 μM to 500 μM. IC_{50} and standard deviation of each compound was calculated by GraphPad Prism version 5.0. %[^{3}H]-Gabapentin uptake at each concentration was normalized relative to %inhibition by BCH[31] at 2 mM, which was set to 100% inhibition.

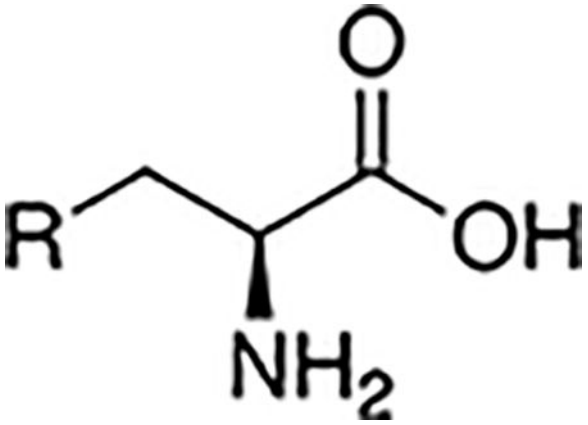
[e] Not determined.

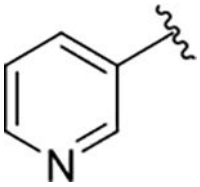
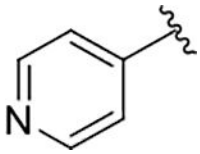
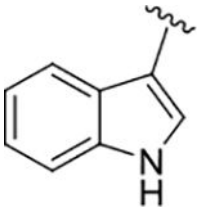
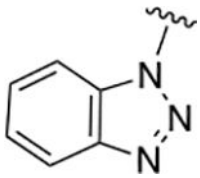
Table 2.

LAT1 *trans*-stimulation and *cis*-inhibition cell assay activity for some naturally occurring amino acids and structural analogs including benzotriazole **13**.[a]



Compound	R	Relative %Efflux	% Inhibition	IC_{50} (μM)
14 (L-Arg)	$NH_2(NH)CNH(CH_2)_2-$	28	49	
15 (L-His)		140	92	110 ± 93
16		85	70	190 ± 25
17		81	74	170 ± 53
18 (L-Phe)		100	85	69 ± 29
19		140	84	160 ± 56



Compound	R	Relative %Efflux	% Inhibition	IC_{50} (μM)
20		130	98	69 ± 17
21		89	99	100 ± 53
22 (L-Trp)		59	79	160 ± 87
13 (L-Trp)		100	59	340 ± 104

[a]Cell assay conditions were as described for Table 1.